

**DIETARY INTAKE, ENVIRONMENTAL TOBACCO
SMOKE, NUTRIENT BIOMARKERS, AND CHRONIC
DISEASE RISK IN UNITED STATES ADOLESCENTS**

by
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ABSTRACT

Environmental tobacco smoke (ETS) exposure and poor dietary intake and quality are known to increase chronic disease risk in adults. Chronic diseases like heart disease, diabetes, and metabolic syndrome (MetS) are major public health problems in the United States. Adolescence is a time period of development of identity and autonomy and increasing importance of peer influence, making teens susceptible to substance abuse and poor dietary intake. The relationships between ETS exposure, dietary intake and quality, nutrient biomarkers, and chronic disease have not been studied together in the adolescent population.

This project utilized data collected as a part of the 2001-2006 National Health and Nutrition Examination Survey (NHANES) – a nationally representative surveillance survey conducted in the United States. Analyses were restricted to non-smokers. General linear models (GLM) were run in STATA to test for differences in diet quality/intake by ETS exposure and to determine whether ETS is related to measures of chronic disease risk in adolescents. Subsequent analyses utilized the Sobel test to determine whether the relationship between ETS exposure and chronic disease risk was mediated by nutrient biomarkers. GLMs with interaction terms were utilized to determine whether dietary intake/quality interacts with ETS exposure in the relationship with chronic disease risk.

These studies found that ETS-exposed adolescents had lower diet quality scores, higher saturated fat intake, and lower nutrient intakes than their unexposed peers. ETS exposure was found to be positively correlated with continuous MetS score. Mediation analyses identified serum folate and trans- β -carotene as potential mediators in the

relationship between ETS exposure and MetS risk. No interaction was identified between dietary intake and ETS exposure in the relationship with chronic disease risk.

This research has demonstrated that adolescents who are exposed to ETS are more likely to have poor diet quality – both of which increase risk for chronic disease.

Findings from this study also demonstrate the negative impact ETS exposure has on chronic disease risk and illustrate some of the potential mechanisms for this relationship.

Longitudinal research is needed to confirm the relationships identified in this study.

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Chapter 1

INTRODUCTION

There has been a dramatic reduction in smoking prevalence over the past few decades which has corresponded with scientific advances in knowledge and policy changes, but smoking is still a significant health problem in the United States. Aside from the well-known relationships with lung cancer and asthma, tobacco smoke contributes to many negative health outcomes including heart disease, diabetes, and metabolic syndrome. Research has shown that these health impacts are not limited to those who smoke, but also impact non-smokers who are exposed to environmental tobacco smoke (ETS). Children and adolescents are particularly vulnerable to the effects of ETS exposure. These same chronic diseases are known to be impacted by diet intake, dietary quality, and nutrient biomarkers. Recommendations have been put forth for dietary intake in order to reduce risk of chronic diseases.

Several studies have demonstrated relationships between smoking or exposure to ETS and poorer diet quality; however, the majority of these studies were conducted in adults and from over 10 years ago. No recent studies have examined the differences in dietary intake and quality between ETS-exposed and unexposed adolescents. While evidence suggests that ETS exposure may negatively impact chronic disease risk, this has not been systematically evaluated in adolescents, and though the mechanisms by which ETS and diet impact chronic diseases have some overlap, these relationships have not been thoroughly explored. Due to these similarities in the pathways to development of chronic disease for ETS and diet, it is also possible that changes in diet could affect this relationship; however, this potential interaction has not been tested in adolescents.

The goal of this study is to understand how ETS exposure, diet, and chronic disease risk are related and the mechanisms behind these relationships. This dissertation is comprised of several chapters. Chapter 2 includes a background and rationale, including a conceptual model, outlining the larger picture of the public health problems and what is known about the relationship between smoking, ETS exposure, diet quality, nutrient biomarkers, and chronic disease; a description of the research aims; details about the data source and variables; and details about the analytic methods.

Chapters 3 through 5 are manuscripts which describe the analyses conducted for the dissertation. Chapter 3 examines the relationship between ETS exposure and dietary intake and quality amongst adolescents. Specifically, this chapter aims to test the hypothesis that adolescents who are exposed to ETS have poorer diet quality as indicated by lower intake of micronutrients and higher intake of saturated fat. While other studies have examined these relationships in adults, there is a paucity of information on this topic specific to adolescents. It is important to understand whether or not poor diet quality and ETS exposure coexist as this would identify a segment of the population with multiple risk factors for chronic disease.

Biomarkers of nutrient status are examined as a potential mediator in the relationship between ETS exposure and chronic disease risk in Chapter 4. First, examining how ETS relates to chronic disease risk in adolescents is an under-studied topic that warrants further investigation. Understanding the mechanisms by which ETS exposure impacts chronic disease risk is also of utmost importance as any identified mechanisms could be targeted by future interventions.

In situations where ETS exposure cannot be eliminated, if differences in dietary intake are associated with reduced risk of chronic disease, this would suggest that improvements in diet intake and quality could mitigate the harmful effects of ETS exposure. These relationships are evaluated in Chapter 5.

Chapter 6 is a concluding chapter with an overall summary of the findings, strengths and limitations, and future directions for research.

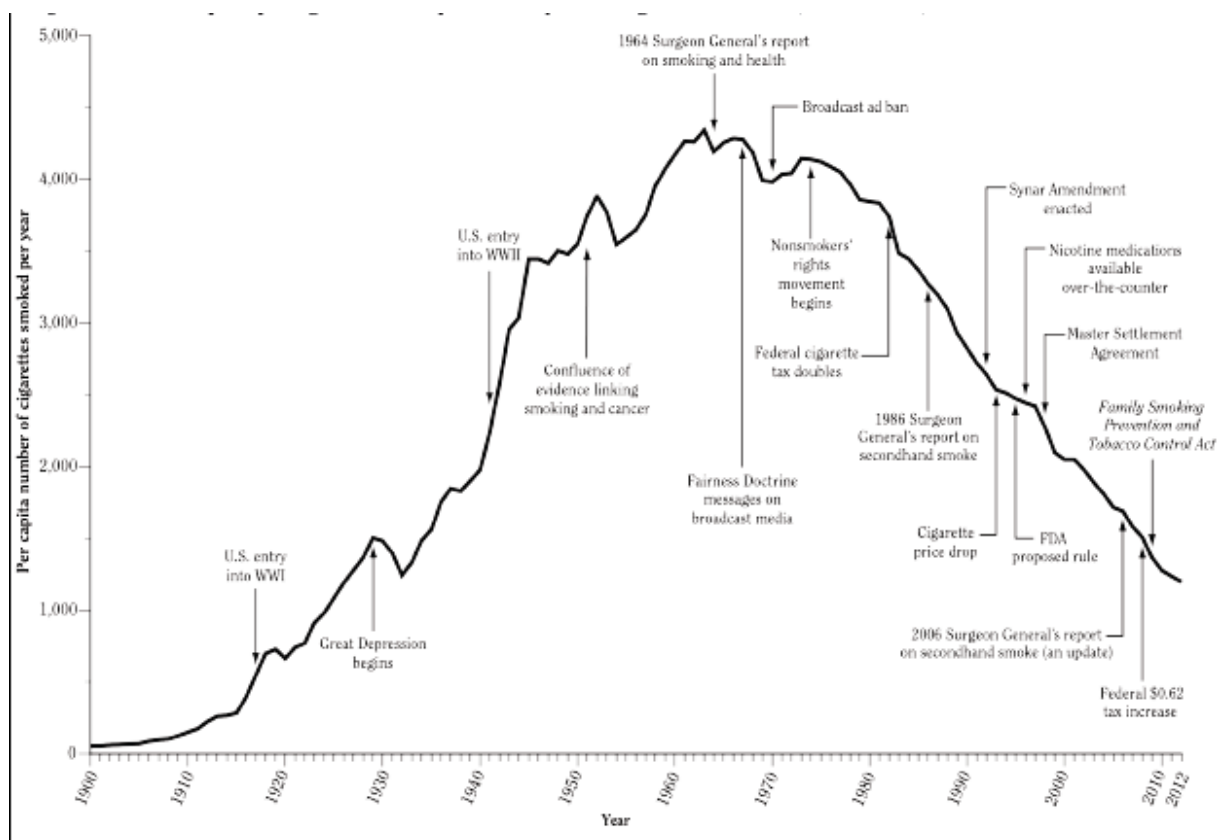
Chapter 2

Tobacco smoking has been a public health concern for centuries. Amongst males born in the United States from 1931 to 1940, at its peak, smoking prevalence was 62 percent and amongst females born during the same time period, the peak prevalence was 45 percent (1). In the 1930's and 1940's most physicians smoked (2). In fact, tobacco agencies utilized physicians in their advertisements with slogans such as, "More doctors smoke Camels than any other cigarette" and "Fredric March says...THIS IS IT 'F&M FILTERS ARE JUST WHAT THE DOCTOR ORDERED!'" (2). Much progress has been made with respect to smoking since these advertisements were made, and the prevalence of smoking has gone down significantly since its peak (see Figure 2.1.). Amongst adults, the prevalence of smoking decreased from over 40 percent in 1965 to just under 25 percent in 1997 (3). In the few years preceding 2011 and 2012, smoking prevalence amongst adults was 28 percent (4). Then, in 2011 and 2012, the prevalence decreased to roughly 25 percent (4).

What caused this reduction in tobacco smoking? Though cigarettes were long thought to cause lung problems and some studies had provided evidence to that effect (documented as early as clinical observations published in 1795 (5)), the study which was considered groundbreaking was published in 1950 by Dr. Ernst L. Wynder and colleagues linking tobacco smoke to lung cancer in a study with over 680 cases of lung cancer (6). It wasn't until 14 years later in 1964 that the Surgeon General released a report which concluded, based on findings from over 7,000 articles, that tobacco smoking was associated with lung cancer and chronic bronchitis in men and women (3). Subsequent to the Surgeon General's report, in 1968 it was determined that the Fairness Doctrine applied to tobacco, which meant that stations broadcasting cigarette advertisements had to also designate air time

to present the harmful effects of smoking and/or anti-smoking messages (3). In 1971, tobacco products were banned from broadcast advertising altogether (3). In the 1980's, shortly after the first Great American Smokeout (a coordinated event where smokers are encouraged to set that date as the day they will quit), federal tobacco taxes were doubled. Other historical milestones include regulation and enforcement of laws restricting minors' access to tobacco, restrictions on smoking in public spaces, and increases in prevention and treatment efforts (3). Throughout this period of scientific discovery and regulatory changes, per capita cigarette consumption declined (see Figure 2.1).

Figure 2.1. Per capita cigarette consumption and historical events from 1900-2012*



*Figure from U.S. Department of Health and Human Services. The Health Consequences of Smoking — 50 Years of Progress: A Report of the Surgeon General. Atlanta, GA: U.S.: Department of Health and Human Services, Centers for Disease Control and Prevention. (7).

Since the discovery that tobacco smoke causes lung cancer, research has established causal relationships between smoking and a plethora of other diseases including: other forms of cancer (pancreatic, bladder, esophageal, throat, mouth, liver, colorectal, breast); multiple forms of heart disease (ischaemic, pulmonary, myocardial) and vascular diseases (cerebral, peripheral); hypertension; arteriosclerosis; and a multitude of lung/respiratory conditions (chronic bronchitis, Chronic Obstructive Pulmonary Disease (COPD), emphysema, asthma, pneumonia, tuberculosis); exposure during pregnancy and orofacial clefts; ectopic pregnancy; erectile dysfunction; macular degeneration; diabetes; suppression of the immune system; rheumatoid arthritis; mortality; and poorer general health (5, 7).

Environmental Tobacco Smoke (ETS) exposure, sometimes referred to as secondhand smoke, is the involuntary inhalation of a combination of exhaled mainstream smoke, and side stream smoke, the non-inhaled smoke produced from burning tobacco (7). ETS exposure can be assessed in multiple ways including subjective measurements, like self-report, and more objective measures like cotinine (a byproduct of nicotine metabolism) concentration and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (or NNAL) (a byproduct of tobacco specific nitrosamines) concentration from a urine, saliva, or blood sample. Before ETS was formally acknowledged as a health risk by the Surgeon General, a non-smokers' rights movement began in the 1970's (3). Then, 22 years after the first Surgeon General's report to acknowledge the negative health effects of smoking, the Surgeon General released a report about the harmful effects of ETS. The 1986 Surgeon General's report on Involuntary Smoking concluded that ETS is a known human carcinogen and highlighted the following health effects of ETS exposure: lung cancer; increased risk of infection, respiratory illnesses and morbidity, and ear infection in children with smoking parents; and decreased pulmonary

function in children and adults (8). The report also indicated that additional research was necessary to determine whether or not ETS increases risk of other cancers and cardiovascular diseases (8).

Research continued to demonstrate harmful effects of ETS, and 20 years after the first Surgeon General Report about ETS exposure, another report was published. The 2006 Surgeon General Report concluded that the health effects of ETS extend beyond the 1986 report to include the following: mortality; increased risk of Sudden Infant Death Syndrome (SIDS); slowed lung growth in children; coronary heart disease in adults (9). The report also found evidence of possible relationships between ETS exposure and preterm delivery, low birth weight, childhood cancer, breast cancer, other cancers, stroke, Chronic Obstructive Pulmonary Disease (COPD) (9). In fact, a meta-analysis conducted for the report indicated a 25-30 percent elevated risk of coronary heart disease due to ETS exposure (9).

While smoking has declined, it has not been eradicated in the United States. Because smoking is still a public health problem, many individuals are also at risk of ETS exposure. Some of the regulatory efforts which have aimed to reduce ETS exposure include indoor smoking bans, prohibition of smoking on airplanes, and workplace smoking restrictions (3). ETS exposure as defined by cotinine concentration between 0.05 and 10 ng/mL was over 50 percent from 1999-2000, was between 37 and 47 percent between 1999 and 2006, and has decreased by almost half to just over 25 percent in 2012; however, 58 million individuals were still exposed in 2011-2012 (10, 11). There are disparities in ETS exposure with children having higher rates of exposure than adults (10).

2.1.1. The adolescent period and smoking/ETS exposure

Despite regulations to decrease smoking in general and efforts to specifically reduce advertising targeting adolescents (3), smoking is still a problem amongst adolescents in the United States. From 2005 to 2013, the smoking prevalence amongst high school students decreased an average of 4.2 percent per year, leading to a prevalence of 15.7 percent (4). While this trend is in the right direction for improvements in public health, the percentage of adolescents who have ever tried a tobacco product is still alarmingly high. In 2013, 46 percent of high school students had tried any tobacco product. In 2014, over 4.5 million middle and high school students were estimated to be currently using any tobacco product (12). Tobacco is likely to remain a public health issue for years to come as the tobacco industry is constantly evolving and developing new products, posing new challenges to tobacco regulation and smoking prevention and cessation efforts. For example, the most commonly used tobacco product amongst adolescents is now e-cigarettes (12).

ETS exposure is also a public health problem amongst United States adolescents. As mentioned earlier, adolescents have higher prevalence of ETS exposure than adults (10). Max et. al. examined NHANES data from 1999-2006 to determine rates of exposure to secondhand smoke. As reported earlier, ETS exposure as assessed by cotinine concentration was between 37 and 47 percent prevalent amongst adults between 1999 and 2006. However, to give a sense of the difference in exposure between adults and adolescents, during the same time period, adolescent exposure was estimated to be between 47 and 63 percent (11).

What are the sources of ETS exposure in the adolescent population? Parental and close friend smoking status and perception that ETS exposure is harmful were both found in international studies to be determinants of ETS exposure in adolescents (13-16). Socioeconomic status (SES) has also been found to be associated with ETS exposure (9, 16).

In a study conducted in a country with high rates of smoking and high rates of ETS exposure, authors found that cotinine concentration was highest on Mondays and postulated that this was due to children being around their smoking parents more on the weekends than weekdays (16). The 2006 Surgeon General's Report on Involuntary Exposure to Smoking indicated that people spend the majority of their time at home, so for those living with a smoker, this is likely the most critical location with respect to exposure (9). Other potential sites for exposure in children include public places, restaurants, and schools.

There are biological reasons why ETS exposure may be more harmful in adolescents than in adults. ETS is metabolized differently in adolescents than in adults. In a study examining NHANES data, the prevalence of ETS exposure in children was higher than adults based on self-report and cotinine concentration. Children (3-11 years) had mean cotinine concentrations ranging from 0.11-0.18 ng/mL compared to 0.09-0.14 ng/mL in adolescents (12-19) and 0.06-0.08 ng/mL in adults (20 and older) (11). In a research study examining the concentrations of cotinine and NNAL and other tobacco-related carcinogens amongst non-smokers in the United States using NHANES data, urinary NNAL was detected in 41% of non-smokers. Authors noted that adolescents aged 12 to 19 years had twice the NNAL as adults (and children aged 6 to 11 had four times as much as adults) (17). Some explanations for the higher concentrations of cotinine and NNAL measured in non-smokers under the age of 19 years as compared with their adult counterparts include smaller size, different respiration, inability to escape exposure, and time spent in the home (17). Willers et al. conducted a semi experimental study on 14 children (aged 4-11 years) and 7 adults after exposure to the same amount of secondhand tobacco smoke (18). Willers measured urinary cotinine and found that the average peak urinary cotinine concentration was 22 mg/L in

children as compared to 13 mg/L in adults ($p=0.005$). Willers explained that children have a higher ventilation rate per unit of body weight, causing them to have a higher dose of nicotine at the same concentration of exposure as adults (18). Furthermore, Willers also suggested that differences may exist in metabolism between adults and children (18).

There are several aspects of the adolescent life stage that make it an important time period for studying tobacco use and exposure. Adolescence is a period of establishing identity and self-perception (19). Identity is composed both of sense of self and continuity of oneself across this period of development but also one's place within the context of larger groups and society as a whole (19). Experimentation and autonomy from parents are key parts of establishment of identity (19, 20) which lend themselves to experimentation with tobacco.

While parents and family continue to influence adolescents, relationships change and the influence of peers reaches its peak during adolescence (19, 21). This process begins in early adolescence, and by late adolescence, the adolescent has developed a separate identity from his or her parents and peers (20). The Theory of Triadic Influence asserts that both direct and interactive factors impact adolescent smoking (22, 23). Three major categories of influence include intrapersonal, interpersonal, and attitudinal. Both parent and friend smoking have been found to influence smoking acquisition and continuation of smoking into young adulthood (23). Psychological factors found to influence smoking behavior in adolescents include having a desire not to be like one's parents and to be like one's peers (23).

From a cognitive development standpoint, adolescence is a period transition from concrete thinking to abstract thought, enhanced reasoning skills and logic, and the ability to

think about thinking (20). The ability to think abstractly lays the framework for adolescents to eventually be able to process potential consequences of their actions as adults, but this new cognitive capability is in its infancy and, therefore, while adolescents may recognize a behavior as risky, they may not yet be able to fully comprehend the long-term effects (20).

These characteristics of adolescence make it an important period of transition for the formation of health behaviors. Will the adolescent identify herself as a member of a group who smokes? Will that peer influence cause her to smoke? Will she begin to integrate smoking into her identity? Does the adolescent have the capacity to think abstractly enough to understand that if she smokes, she is putting her health at risk? Can smoking be a form of establishing autonomy from one's parents?

Cigarette smoking in children and adolescents is a great concern due to the addictive nature of cigarettes and the finding that children and adolescents who smoke are more likely to smoke as young adults and have greater risk of other negative health outcomes (23, 24). Health behaviors developed in adolescence track into adulthood. This has been demonstrated specifically in longitudinal studies of smoking which have found that adolescents who smoke are more likely to smoke as young adults and adults than adolescents who do not smoke (25-27).

2.1.2. Chronic disease in the United States

Cardiovascular disease (CVD) and diabetes (T2DM) are two of the leading causes of death in the U.S. causing 597,689 and 69,071 deaths in 2010 respectively (28, 29). Data from the National Health Interview Survey in 2009 demonstrated that in a sample of 227,371 adults, 11.8 percent had CVD, 24.9 percent had hypertension, 2.6 percent had experienced a stroke, and 9.0 percent had diabetes (30). Data from 3,423 adults in the National Health and

Nutrition Examination Survey (2003-2006) indicated a 34 percent prevalence of metabolic syndrome (MetS) (a disease associated with increased risk for CVD and diabetes) (31).

One major preventable contributor to chronic disease is tobacco. From 2005-2009, 397,840 males and 422,330 females ages 35 years and older died from cardiovascular and metabolic diseases (7). Of those deaths, 95,600 and 65,000, respectively, could be attributed to smoking (7). ETS alone accounts for approximately 41,280 deaths annually to non-smokers due to heart disease and lung cancer (7).

Chronic disease develops across the lifespan. The Developmental Origins of Health and Disease hypothesis argues that chronic disease development begins as early as the fetal environment – that adverse conditions in utero can negatively impact phenotype leading to increased susceptibility to chronic disease (32). Precursors to cardiovascular disease such as increased local pulse-wave velocity, left ventricular mass, left atrial volume, and carotid intima-media thickness have been observed in obese adolescents (33, 34).

Prevalence and characterization of chronic disease differs between pediatric and adult populations. MetS is clearly defined in adults; however, it has been less clearly defined in children and adolescents. Furthermore, the prevalence of MetS, like many other chronic diseases which may develop over an extended period of time, is lower in children and adolescents (35). Therefore, it is especially important to use a continuous measure of MetS for children and adolescents when examining determinants of disease in order to have sufficient power to detect relationships. Other arguments for a continuous score are that it is less susceptible to error than dichotomous characterization of MetS and it is a more sensitive measure (35). In a pediatric population, the components of MetS must be age and gender standardized and, if possible, standardized for maturation status (35). Instead of using fasting

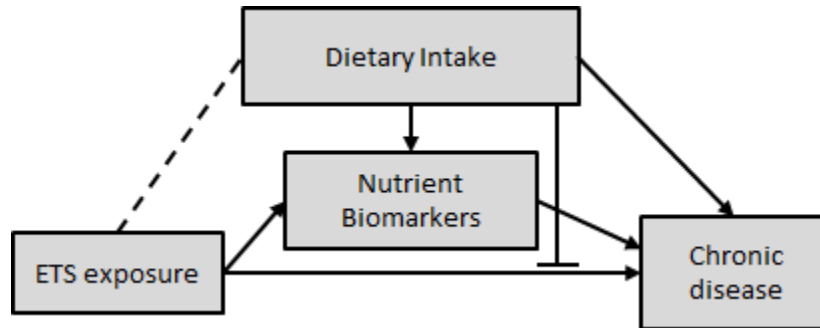
plasma glucose as is done with adult populations, it is better to utilize homeostasis assessment model of insulin resistance (HOMA-IR) because most children have normal fasting plasma glucose (36). HOMA-IR is an assessment of insulin sensitivity ($22.5/[\text{fasting insulin} \times \text{fasting glucose}]$) (37). Furthermore, in order to incorporate both systolic and diastolic blood pressure, mean arterial pressure ($\text{MAP} = ((\text{systolic blood pressure} - \text{diastolic blood pressure})/3) + \text{diastolic blood pressure}$) is ideal for use in the calculation of a continuous MetS score for pediatric populations (36).

The definitions of hypertension and dyslipidemia are different for adults and adolescents. For adults, cutoff values are used to delineate absence of hypertension from prehypertension and hypertension. For children, prehypertension, stage 1 hypertension, and stage 2 hypertension are based on blood pressure percentiles for age and sex (38). While cutoffs are used for diagnosis of high cholesterol in children and adults, the cutoff values are different, and there is a “borderline hypercholesterolemia” category for children which does not exist for adults (39). Finally, there is a Framingham CVD Risk Score that can be used in adults but not in children.

CVD, T2DM, and MetS are also prevalent amongst adolescents in the United States. NHANES data from 2,456 adolescents aged 12 to 19 years demonstrated an 8.6 percent prevalence of MetS. Approximately half of the sample had one of the criteria for the disease. In this sample, 6.9 percent had blood pressure greater than or equal to the 90th percentile and 14 percent had fasting plasma glucose greater than or equal to 100 mg/dL (40). Using NHANES data from 1999 through 2006, Ford et. al. found that 8.6 percent of adolescents aged 12-17 years had elevated total cholesterol using the National Cholesterol Education Program guidelines (41).

Many aspects of the relationship between ETS exposure and chronic disease risk have not been thoroughly evaluated. The following sections of Chapter 2 provide the background behind some of those still unexplored relationships. A visual depiction of these yet unexplored relationships – a conceptual model – is presented in Figure 2.2.

Figure 2.2. Conceptual Model



2.1.3. Mechanisms by which ETS impacts chronic disease risk

ETS exposure is associated with the development of CVD in both adults and children (42). The effects of ETS are a result of a combination of smoke components including carbon monoxide, nicotine, polycyclic aromatic hydrocarbons, and other elements. In particular, exposure to ETS induces oxidative stress when carbon monoxide displaces and competes with oxygen for binding sites on red blood cells, which reduces the blood's ability to deliver oxygen to the heart and compromises the myocardium's ability to use oxygen to create adenosine triphosphate (43). Other mechanisms that may also explain this relationship include effects on inflammation, platelet aggregation, and endothelial dysfunction (44). And, with these mechanisms, research has found that ETS exposed individuals appear to have disproportionately increased concentrations of two biomarkers of CVD risk: fibrinogen and homocysteine (43, 45, 46). In a cross-sectional study of 205 young adult females (18-22 years old), investigators examined a host of lifestyle characteristics and their associations with intima media thickness – a known risk factor for CVD. In multivariate analysis, the

investigators found that exposure to ETS was statistically significantly associated with high intima media thickness – a known risk factor for CVD (OR: 1.5, $p=0.018$) (47).

ETS exposure also increases risk for T2DM and MetS. A population-based cohort study examined the effect of passive and active smoking on T2DM incidence and found that among never smokers, subjects exposed to ETS had increased T2DM risk (48). A growing body of evidence indicates that a possible biological explanation for this relationship is the positive association between ETS exposure and insulin resistance (49, 50). With respect to risk for developing MetS, in a recent cross-sectional study, researchers found that exposure to ETS was independently and significantly associated with enhanced risks of MetS and its individual components (51).

Adolescents exposed to ETS are also at higher risk for MetS. Weitzman and colleagues examined the relationship between ETS exposure and MetS amongst 3,211 12-19 year old adolescents in NHANES data from 1988 to 1994. Using multivariable logistic regression including adjustment for age, race/ethnicity, gender, poverty, parent history, and region, ETS exposure was associated with a 4.7 fold increased risk of MetS ($p=0.003$) (50). This relationship remained in subsequent analyses examining only overweight and obese youth. Authors argued that the established association between smoking and insulin resistance could explain these findings.

It is evident that ETS is associated with reductions in concentrations of antioxidants in those who are exposed when compared to those who are unexposed (42, 52-57). Also apparent is the relationship between low concentrations of antioxidants and increased risk of chronic disease (52, 54, 58, 59). This supports the theory that nutrient biomarkers act as a mediator in the relationship between ETS and chronic disease such that ETS causes

reductions in antioxidants which, in turn, promotes the development of chronic disease. However, this mediating relationship has not been explored in the adolescent population.

Specifically, ETS is associated with decreased concentrations of certain nutrient biomarkers including folate, ascorbic acid, β -cryptoxanthin, α -carotene, and β -carotene. ETS-exposed children have lower nutrient biomarker concentrations than unexposed children even after adjusting for dietary intake (52). The evidence of the association between lower serum and red blood cell folate and ETS exposure stems from analysis of NHANES data in adults ($n=15,564$) (53), even after stratification by folate intake, 37 and in children (2,218 children ages six to 18) (57).

Several studies demonstrated negative associations between ETS exposure and ascorbic acid concentration. Tribble et. al. recruited a sample of 141 premenopausal, healthy (no T2DM or CVD) women aged 25-45 years to examine the effects of smoking and ETS on plasma ascorbic acid. Plasma ascorbic acid was statistically significantly lower in women who smoked or who were exposed to ETS than non-smoking women who were not exposed to ETS (55). NHANES data has confirmed this association amongst children ($n=2,968$) aged four to 18 years. Furthermore, Strauss et. al. reported a linear relationship between cotinine concentration and serum ascorbic acid and a dose-dependent relationship such that higher concentrations of ETS exposure were associated with lower concentrations of ascorbic acid (56). Associations between ETS exposure and ascorbic acid concentrations often persist after adjustment for intake; however, most articles do not report whether intake differed between ETS exposed and unexposed groups (60).

Carotenoid concentrations are also lower in the presence of ETS exposure. In a study of non-smoking women, after controlling for dietary intake, plasma β -carotene was

significantly lower in non-smoking women who lived with a smoker when compared to women who did not live with a smoker (60). This relationship also exists in children and adolescents. Investigators examined data from children enrolled in the Diabetes Autoimmunity Study in the Young (DAISY) (n=257) and discovered that ETS exposure was associated with lower β -cryptoxanthin, α -carotene, and β -carotene, regardless of intake (52). Analysis of NHANES data confirms this relationship in children and adolescents (n=2,218; ages 6-18 years) (57).

2.1.4. Diet quality and chronic disease

Like ETS exposure, dietary intake is known to impact chronic disease development. As recognized by the Dietary Guidelines for Americans, produced by the United States Department of Agriculture and the Department of Health and Human Services as recommendations for dietary intake which will promote health and reduce the risk of chronic disease, decreased intakes of sodium, saturated fatty acids, solid fats, cholesterol, trans-fatty acids, added sugar, and alcohol and increased in intake of fruits, vegetables, whole grains, lean meats and protein sources, seafood, oils, milk, and milk products are associated with reduced risk of chronic disease (61). Better scores on the alternate Healthy Eating Index (aHEI) are associated with lower concentrations of c-reactive protein (62, 63).

Studies, including longitudinal cohort studies, investigating the aHEI have found that higher aHEI scores are associated with lower 10-year coronary heart disease risk, lower incidence of cardiovascular disease events and heart failure, and lower incidence of T2DM, and that the aHEI score was a stronger predictor of CVD risk than the HEI score (63-66).

Evidence from randomized controlled trials has demonstrated that the DASH diet can reduce chronic disease risk. The DASH diet, or Dietary Approaches to Stop Hypertension, is

high in fruits, vegetables, and low- or non-fat dairy, incorporates whole grains, includes beans, nuts, seeds, fish, and poultry, and vegetable oil, and restricts red meat, sweets, sodium, and sugar-sweetened beverages (67). The goal for macronutrient composition is to have roughly 27 percent calories from fat, six percent from saturated fat, 55 percent from carbohydrates, and 18 percent from protein (67). The diet aims to restrict cholesterol to 150 mg, sodium to less than 2,300 (or in more restricted versions, 1,500) mg, and to include 4,700 mg potassium, 500 mg magnesium, 1,250 calcium, and 30 grams of fiber (based on a 2,000 kcal diet) (67). In randomized controlled trials, the DASH diet, when compared to the control group, has found to be associated with higher HDL and reductions in weight, blood pressure, and triglycerides, fasting plasma glucose, and c-reactive protein, LDL, and apolipoprotein (68, 69). Another experimental study found that in obese individuals, antioxidant capacity increased with the DASH diet, which could explain some of the reduction in chronic disease risk seen with this diet (70).

Intake of specific types of fats, such as polyunsaturated fatty acids, very long chain n-3 fatty acids (VLC n-3), and monounsaturated fatty acids, have been associated with multiple positive health effects such as decreases in triglycerides, LDL cholesterol, inflammation, blood pressure, and effects on membrane structure, coagulation, lipoprotein oxidation, fibrinolysis, endothelium, and blood clotting (71-73). Other fats, such as hydrogenated fats, have negative health effects including increased c-reactive protein and decreased interleukin-8, increased reactive oxygen species, tumor necrosis factor, and promotion of atherosclerosis and inflammation, along with adverse effects on endothelial function and plaque rupture (74-76). Higher fiber consumption is associated with lower concentrations of c-reactive protein (77).

Many of the biomarkers of CVD risk also increase the risk of morbidity in those with T2DM. Homocysteine, in particular, is a biomarker with clearly established relationships with both CVD and T2DM. Some examples of dietary characteristics associated with homocysteine include high protein/low carbohydrate diets, polyunsaturated fatty acid, micronutrient, and folate intake (78-88). Insulin, glucose, and HbA1c are biomarkers of T2DM risk which are affected by ETS exposure and dietary intake.

Evidence from the chronic disease literature suggests an overlap in the pathways through which dietary intake and ETS exposure affect health outcomes (43-46, 71-96). In particular, dietary intake affects a number of the same biomarkers (or risk factors) of CVD as does ETS exposure. Cardiovascular disease, diabetes, and metabolic syndrome have some shared biological etiologies, including inflammation, oxidative damage, and oxidative stress. As previously demonstrated, ETS increases oxidative stress and decreases concentrations of antioxidants in the blood whereas dietary intake can either increase antioxidant capacity or increase oxidative stress depending on dietary quality (i.e., micronutrient and fat content).

Homocysteine contributes to chronic disease risk by promoting oxidative injuries to arteries, damaging vasculature, inhibiting anti-coagulants, and facilitating smooth muscle proliferation (97). As mentioned before, ETS exposure is also associated with elevated homocysteine. Diet affects homocysteine as well. One nutrient that impacts homocysteine is folate/folic acid – insufficient folate/folic acid intake can cause elevated homocysteine concentrations (98).

2.1.5. The adolescent period and diet

The developmental characteristics of the adolescent life stage described earlier have an impact on food choices as well (99). Adolescence marks a time when children seek

autonomy and control over their food choices. They are also influenced by peer food selections, and these factors may lead to poor choices. Adolescents are at risk for nutritional deficiencies due to poor diet quality (fast food, sugar, and soft drink consumption and insufficient dairy and fruits and vegetables) (99). Due to growth occurring during adolescence, demand for energy and nutrients is higher, especially amongst adolescent boys (99). Vitamin requirements approximate adult needs with the exception of vitamin B6 and niacin which have greater requirements, and iron, calcium, and phosphorus (and magnesium for females) needs exceed adult requirements (99).

Data from the National Health and Nutrition Examination Survey have been utilized to examine whether or not adolescents meet dietary recommendations. Analysis of the 1999-2002 data demonstrated that only nine percent of adolescents consumed five or more fruits or vegetables in a day (100). In 2003-2004, only 6.2 percent of adolescents met recommendations for fruit intake and 5.8 percent met recommendations for vegetable intake (101). There is evidence that fruit and vegetable consumption decreases during this life stage (102). Studies that have examined trends in child and adolescent diet in the United States demonstrate that vegetable intake decreased from 1989 to 2010 and fruit intake had declined early during this time period but recently increased from the 2003-2006 period to 2009-2010 (103). Other similar studies have found no change in vegetable intake in recent years and that despite increases in fruit intake amongst children and adolescents, in 2009-2010, adolescents were still not meeting the Healthy People 2020 goals for fruit and vegetable consumption (104).

The evidence and opinions regarding whether dietary patterns in adolescence track into adulthood are mixed. Some argue that dietary patterns do track from adolescence to

adulthood and that this means that dietary intervention during adolescence is critical. Consumption of fruits, vegetables, and sugary foods has been found to track from adolescence to young adulthood (105). When analyzed as a proportion of total food intake, a longitudinal study found that fruit, vegetable, potato, cereal, bread, fish, meat, and alternatives tracked over a 20 year period but found that dairy and sugary food intake did not track over time (106).

2.1.6. Nutrient biomarkers and chronic disease

Reduced concentrations of nutrient biomarkers have been associated with chronic disease. There is a biological basis for this relationship. Some micronutrients (i.e., retinol, α -carotene, and β -carotene) are required for epithelial cell differentiation and cell signaling and communication. Other micronutrients act as antioxidants which shield cell membranes from damaging effects of lipid peroxidation (52). Micronutrients in the blood can reduce cell proliferation and protect against platelet adhesion (52). Epithelial cell differentiation, cell signaling and communication, insulin production, oxidation, cell proliferation, and platelet adhesion are all processes which can affect risk of CVD, T2DM, and MetS (59).

Roberts and Sindhu published a review of the relationship between oxidative stress and MetS (59). The authors defined oxidative stress as an imbalance between reactive oxygen species and antioxidant capacity and described how the antioxidant capacity is composed of both endogenous and dietary antioxidants (i.e., vitamin C, E, β -carotene, and phytochemicals). The literature demonstrates that oxidative stress is associated with insulin resistance, adiposity, total body fat, waist circumference, endothelial dysfunction, lipoprotein atherogenicity, and arterial blood pressure – all components of either CVD, T2DM, or MetS (59).

In studies comparing those with chronic diseases to those without, nutrient biomarkers, especially those of antioxidants, are often lower amongst individuals with the disease than those without the disease. A study comparing vitamin C concentrations in patients with peripheral arterial disease (PAD), hypertension without PAD, and healthy individuals found that PAD patients had statistically significantly lower vitamin C concentrations than hypertensive and healthy individuals and that this group was the only group to have any subclinical vitamin C deficiency (14% of PAD patients) (54). A study of NHANES participants found that participants with MetS had statistically significantly lower vitamin C and carotenoid concentrations than those without MetS (58). MetS has been associated with lower serum vitamin C and α -tocopherol concentrations (59). Evidence for the causal relationship between oxidative stress and chronic disease is not entirely based on observational studies. In a randomized controlled trial, antioxidant supplementation administered to patients with essential hypertension resulted in improvement in flow-mediated dilation of the brachial artery, reductions in arterial stiffness, and reductions in insulin resistance (59).

2.1.7. Diet quality and nutrient biomarkers

Dietary intake of micronutrients, including antioxidants, is associated with blood concentrations of antioxidants. Higher intakes of vitamin D, retinol, α -carotene, β -carotene, β -cryptoxanthin, lutein and lycopene are associated with higher concentrations of plasma 25-hydroxyvitamin D, plasma retinol, plasma α -carotene, plasma β -carotene, plasma β -cryptoxanthin, plasma lutein and plasma lycopene, respectively, in children aged nine months to eight years (52). Amongst over 500 two to 13 year olds in Puerto Rico, plasma ascorbic acid concentrations were associated with vitamin C intake (107).

2.1.8. Diet quality and smoking/ETS

There is evidence in the literature that smoking is related to poor diet quality amongst adults and adolescents (108-112). Adult smokers have lower intakes of vitamin C, fiber, dairy, fruits and vegetables, more fast food, and overall poorer diet quality (111, 113). Adolescent smokers consume fewer fruits, vegetables, dairy, and grains and more soft drinks (109, 114). Furthermore, in a study conducted amongst a diverse sample of 4,746 adolescents in Minnesota from 1998-1999, authors found that adolescent smokers had lower intake of fiber, calcium, iron, zinc, vitamin A, vitamin C, and folate than non-smokers (109).

Some studies have also found a correlation between ETS exposure and poor diet quality (115-121); however, this literature is sparse and from 13-27 years ago. There is also some evidence to suggest that adults exposed to ETS have poorer diet quality than unexposed individuals (less fruits, vegetables, milk, lean meats, complex carbohydrates, and beans; more fried food, fat, and cholesterol; lower iron, β -carotene, and fiber) (39, 115-122). In 1989, using data from over 3,000 adult members of Kaiser Permanente who had a health checkup, Sidney et. al. found that nonsmokers exposed to ETS had statistically significantly lower intakes of carotene than nonsmokers who were unexposed to ETS (123). Amongst nonsmokers, those who live with smokers have a less healthy dietary intake than nonsmokers who do not live with a smoker (124). Household ETS exposure has been associated with lower micronutrient content in the diet, and worksite ETS exposure is associated with lower vitamin C, fruit, and vegetable intake than those who are not exposed to ETS (121).

If, in fact, dietary intake can exacerbate the negative effects of ETS on chronic disease risk, then a tendency for ETS exposed individuals to have less healthy diets would warrant targeted dietary interventions for this population. Furthermore, this would be a

special consideration for confounding when investigating the potential mediating effect of nutrient biomarkers in the relationship between ETS and chronic disease.

2.1.9. Summary

While smoking has declined in the United States, it has not been eliminated. Smoking contributes to several of the leading causes of death in the United States – specifically cardiovascular disease and diabetes. Similarly, ETS exposure increases risk of chronic disease as well. Adolescents are in a developmental stage where they have not yet fully developed the ability to think abstractly to comprehend long-term consequences of their actions and they are trying to establish autonomy from their parents. This makes them susceptible to initiation of substance use. Adolescents are also at risk of poor diet quality in the United States.

As demonstrated above, the relationships between dietary intake and chronic disease risk, ETS and chronic disease risk, dietary intake and antioxidant concentrations, ETS and antioxidant concentrations, and antioxidant concentrations and chronic disease risk are all well established in the literature. However, insufficient research has been conducted to examine the bigger picture of how these factors work together to influence development of chronic disease. It is important to understand whether or not ETS exposed adolescents have poorer diet quality because if that is the case, this is a high risk group to target for prevention efforts. This study will bridge this gap in the literature by evaluating whether such clustering of health exposures and behaviors exists amongst the U.S. adolescent population.

Identifying the biological mechanisms by which exposures impact health is one of many critical factors for establishing causal relationships. While evidence suggests that part of the negative impact ETS exposure has on chronic disease risk is via reductions in nutrient

concentrations, this has not been systematically explored. This study began investigating this possibility and provides evidence for future, longitudinal studies to examine this relationship further.

A recent review of literature suggested that diet may have a partial interaction with ETS exposure/smoking in the development of these diseases, but that further research is necessary to establish this relationship (125). The need for an examination of these relationships within the same population at the same time is striking. By assessing the influence of dietary characteristics on the relationship between ETS exposure and risk for developing CVD, T2DM, and MetS, using a large, nationally representative dataset, the National Health and Nutrition Examination Survey (NHANES), this study will address this gap in the literature. Because chronic diseases often take time to develop, adolescents have lower prevalence of chronic disease. Therefore, prevention is key in this population. In order to implement cost-effective disease prevention, identification of populations at highest risk is often necessary. If dietary intervention to improve diet quality can mitigate the harmful effects of ETS exposure, this would be a strategy which could be employed to reduce chronic disease risk amongst the adolescent population.

2.2 RESEARCH AIMS

The goal of this study is to gain a better understanding of how ETS and chronic disease are related in adolescents. Specifically, this study seeks to understand differences in diet by ETS exposure and how diet affects the relationship between ETS and chronic disease. In this investigation, nutrient biomarkers and dietary intake characteristics were examined. The nutrient biomarkers included in this research are antioxidant concentrations which have been negatively associated with the occurrence of chronic diseases. Folate concentrations

will also be examined as folate deficiency is associated with increased homocysteine, and increased homocysteine is associated with increased chronic disease risk. The dietary characteristics proposed are modifiable and are known to affect CVD, T2DM, and MetS (43-46, 71-77, 79, 81, 83, 85-92). If dietary intake interacts with the effect of ETS on chronic disease risk, interventions targeted toward these dietary characteristics could be developed. For example, if the alternate Healthy Eating Index 2010 (aHEI 2010) is found to interact with the effect of ETS on CVD, it would suggest that ETS-exposed individuals should not only be targeted for interventions to reduce ETS exposure, but also for dietary interventions aimed at achieving a dietary pattern which aligns with aHEI 2010 characteristics. Not only will this study reveal whether dietary intake interacts with the effect of ETS on chronic disease risk, but it may suggest ways in which the harmful effects of ETS on chronic disease risk could be mitigated through specific dietary intervention.

AIM 1: Examine the relationship between ETS and dietary intake and diet quality in adolescents.

H1: Greater exposure to ETS as measured by self-report and cotinine concentrations will be associated with lower scores on measures of dietary quality (alternate Healthy Eating Index 2010 and DASH diet).

H2: Greater exposure to ETS will be associated with higher intake of saturated fats and lower dietary intake of micronutrients (folate, vitamin C, β -carotene, and α -tocopherol) and fiber.

AIM 2: Determine whether nutrient biomarkers mediate the relationship between ETS and chronic disease risk in adolescents.

H1: Greater exposure to ETS will be associated with higher continuous metabolic syndrome score and higher prevalence of hypertension, dyslipidemia, and T2DM.

H2: Greater exposure to ETS will be associated with lower red blood cell folate, serum folate, plasma ascorbic acid, and trans- β -carotene concentrations.

H3: Lower concentrations of red blood cell folate, serum folate, plasma ascorbic acid, and trans- β -carotene will be associated with higher continuous metabolic syndrome score and higher prevalence of hypertension, dyslipidemia, and T2DM.

H4: Part of the relationship between ETS and chronic disease risk (continuous MetS risk score, hypertension, dyslipidemia, and T2DM) will be mediated by red blood cell folate, serum folate, plasma ascorbic acid, and trans- β -carotene concentrations.

AIM 3: Determine whether dietary intake moderates the relationship between ETS and chronic disease risk in adolescents.

H1: Higher scores on measures of quality/patterns of dietary intake (alternate Healthy Eating Index 2010, DASH diet) will attenuate the positive association between ETS exposure and continuous MetS risk score, hypertension, dyslipidemia, and T2DM.

H2: Greater intake of saturated fats and lower dietary intake of micronutrients (folate, vitamin C, vitamin A, and vitamin E) and fiber will exacerbate the positive association between ETS exposure and continuous MetS risk score, hypertension, dyslipidemia, and T2DM.

Overall, the proposed project will improve scientific knowledge about the relationships between ETS, antioxidant concentrations, and chronic disease in adolescents, and will help determine whether dietary intake can modify the relationship between ETS and chronic disease. These findings would have implications for public health recommendations and regulations. It may potentially identify target populations (ETS-exposed individuals) for strategic dietary intervention.

Examining the effects of ETS, dietary intake, antioxidant concentrations, and chronic disease risk amongst adolescents is imperative given the impact of these findings. If dietary intake can combat the deleterious effects of ETS on chronic disease risk in adolescents, it would warrant interventions which could prevent the development of disease over the life course.

2.3 DATA SOURCE

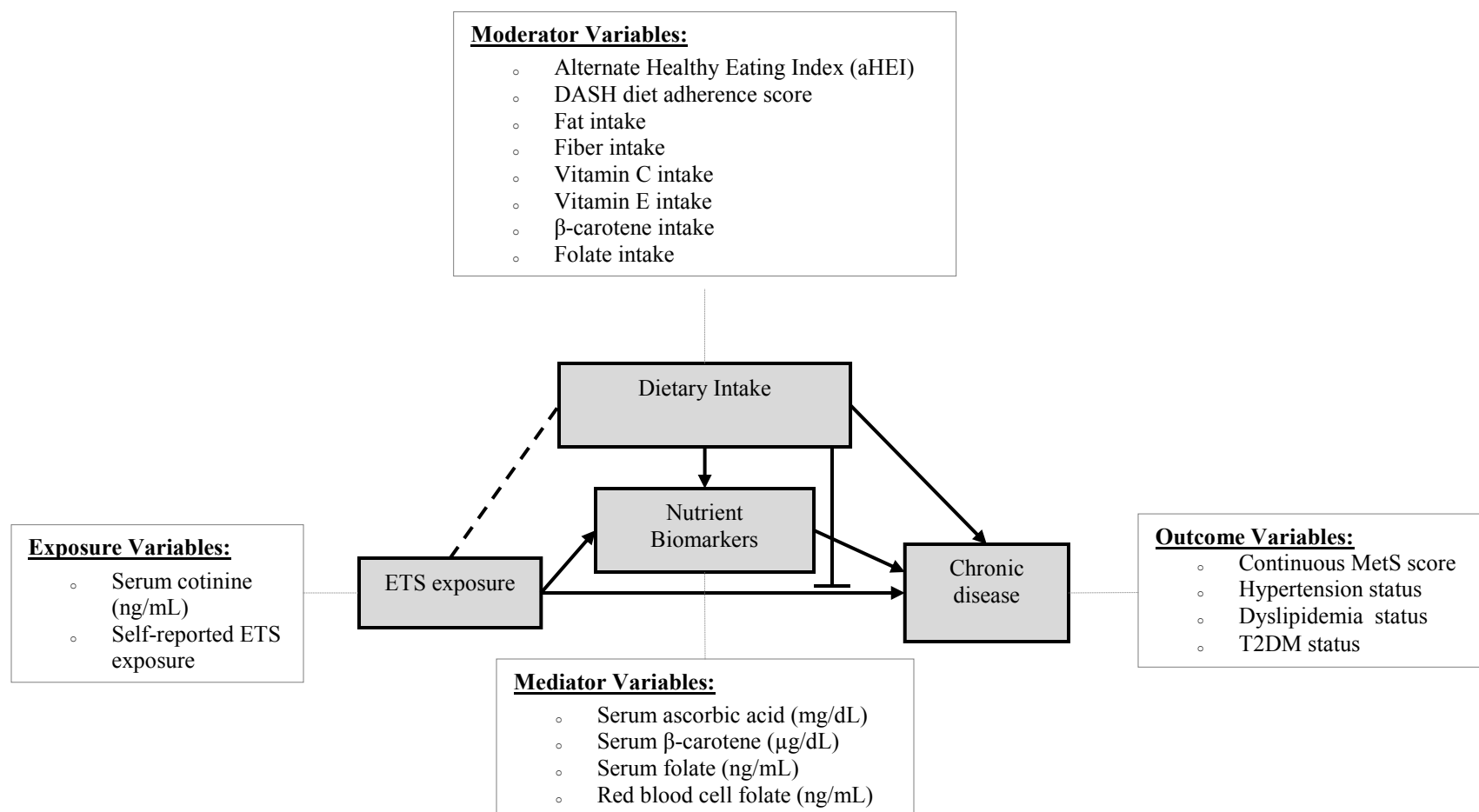
This project was carried out using data from the National Health and Nutrition Examination Survey (NHANES) years 2001-2006 (126). NHANES is an ongoing, cross-sectional, national surveillance survey designed to assess the health and nutritional status of a representative sample of adults and children in the United States (127). The design of NHANES includes oversampling of African Americans, Hispanics, and individuals over 60 years of age to allow for subgroup analyses. The NHANES sample includes children, adolescents, and adults. The survey combines interviews (self-report responses), dietary recall, and physical examinations, with the collection of biologic samples (urine, blood) (127). This dataset includes all of the necessary dietary data, biomarkers of exposure, biomarkers of dietary antioxidants, and biomarkers and measures of chronic disease risk needed to test the hypotheses of this study.

Data files from NHANES 2001-2002, 2003-2004, and 2005-2006 containing the variables needed for this study were downloaded from the NHANES website and merged into one file using STATA 13.1. Data coding and cleaning and statistical analyses were completed in STATA 13.1 (128).

In order to be included in this study, participants were required to have data for the exposure (cotinine concentration and self-report) variables and other variables depending on the analyses (i.e. mediating variables (nutrient biomarkers), moderating variables (24-hour recall data), and outcome (fasting blood panel, blood pressure, and waist circumference data)). Participants aged 12 through <20 years were included in the analyses.

Current smokers were excluded from this study as there is no way to distinguish the effects of smoking from the effects of ETS. Pregnant women were also excluded because the interpretation of many blood measures differs during pregnancy as compared to other stages of life.

Figure 2.3. Variables of interest and data collection procedures



Exposure variables

ETS exposure was assessed in NHANES 2001-2006 using a combination of self-report and cotinine concentrations. Self-reported tobacco smoke exposure was assessed by determining whether anyone who lives in the household smokes and, if so, the number of cigarettes smoked inside the home (129-131). While self-reported ETS exposure is important to measure, it is also important to assess ETS using more objective measurements due to the stigma associated with smoking. Smoking prevalence from a study was reported earlier based on an objective measure – cotinine concentration. This study used NHANES data as well and reported the self-reported ETS exposure prevalence. In adults, self-reported secondhand smoke exposure (workplace and home combined) ranged from roughly 13 to 16 percent as compared to 37 to 47 percent based on cotinine concentration. For adolescents aged 12-19, self-reported exposure ranged from approximately 15-23 percent vs. 47-63 percent exposure based on cotinine concentration (11).

Cotinine is the major metabolite of nicotine with a half-life of approximately 16-18 hours after smoking ceases (132). It is formed from oxidation in the liver and about 70-80 percent of nicotine is converted to cotinine (132). Cotinine can be measured in the saliva, urine, plasma, and serum of smokers and environmentally exposed non-smokers. Using NHANES data, Mannino et. al. demonstrated that the average number of cigarettes smoked in a household was a statistically and clinically relevant predictor of serum cotinine concentrations in children (133). Cotinine concentration can be used to distinguish smokers from non-smokers in smoking cessation trials and in epidemiologic studies. Various cotinine concentration cutoffs have been proposed to distinguish active smokers from non-smokers.

A certified phlebotomist performed blood draws in the Mobile Examination Center (MEC) for NHANES 2001-2006. Participants were excluded from blood draws if they had hemophilia, received chemotherapy four weeks or fewer prior to the exam date, or if other medical conditions were present on their arms (134). NHANES 2001-2006 had extensive quality control plans in place. Samples from participants were run twice when possible and, if the results were in conflict, the sample was run a third (and fourth) time. Calibration materials which qualify under Clinical Laboratory Improvement Amendments (CLIA) were utilized, Beckman Coulter® representatives performed quality control checks, an Interlaboratory Quality Assurance Program (IQAP) was in place which compared results with those of other laboratories, 5C® Cell Control was used, a proficiency testing program was in place (College of American Pathologist), freezers were kept between -18 and -23° Celsius, and regular automated checks were integrated into the Integrated Survey and Information System (ISIS) (135). Dry runs were conducted during which blind, split samples were analyzed in the Mobile Examination Center (MEC). Specific rules applied when running specimens for NHANES called the Westgard rules which are described in the quality control protocols for the laboratories. The National Center for Health Statistics (NCHS) and Westat received summary statistics related to quality control quarterly for review (135-137).

Blood samples collected in red-top tubes were analyzed in the MEC using blind split samples. Contract laboratories performed repeat testing randomly on two percent of the samples collected. Test results below a lower detection limit of any test were replaced by the detection limit value divided by the square root of two (135, 137, 138).

In NHANES 2001-2006, serum cotinine concentrations were determined using ID HPLC-APCI MS/MS (isotope dilution-high performance liquid chromatography /

atmospheric pressure chemical ionization tandem mass spectrometry) by the Division for Environmental Health Laboratory Sciences at the Centers for Disease Control and Prevention (136, 139, 140). Serum cotinine was derived from blood samples taken from participants aged three years and older. In 2001-2002, the limit of detection for cotinine was 0.05 ng/mL; however, during the 2003-2004 cycle and through 2005-2006, the limit of detection for cotinine changed to 0.015 ng/mL (136, 139, 140).

Mediator variables

The mediating variables of interest for this study were concentrations of serum vitamin C, serum β -carotene, serum α -tocopherol, serum folate, and red blood cell folate. Serum folate was measured on participants ages one and older using the Bio-Rad Laboratories radioassay kit by the Centers for Disease Control and Prevention Division of Environmental Health Laboratory Sciences. The limit of detection was 0.1 ng/mL for serum folate and 20 ng/mL for red blood cell folate (136).

Serum vitamins A, E, and carotenoids were measured on participants ages six and older using liquid chromatography with multi-wavelength photodiode-array absorbance detection. In 2001-2002 and 2005-2006, the National Center for Environmental Health performed testing; whereas, in 2003-2004, Craft Technologies performed testing. Vitamins A, E, and carotenoids were measured on participants three and older in 2001-2002 but only on participants ages six and older in 2003-2004 and 2005-2006. For serum vitamin A, the detection limit was 1.03 $\mu\text{g/dL}$, trans- β -carotene was 0.8 $\mu\text{g/dL}$, and for serum vitamin E it was 40.7 $\mu\text{g/dL}$ (141). Vitamin C was analyzed by the National Center for Environmental Health and the Centers for Disease Control and Prevention using isocratic HPLC with electrochemical detection at 650 mV on participants ages six years and older (142).

Moderator variables

The moderating variables of interest included the aHEI 2010 (62, 63), DASH diet adherence score (143), saturated fat intake, fiber, vitamin C intake, vitamin E intake, β -carotene intake, and folate intake. These were derived using the 24-hour dietary recall data.

NHANES 2001-2006 conducted 24-hour dietary recalls using the Automated Multiple Pass Method (AMPM) (144). Respondents reported all foods consumed during the previous day from the time the individual woke up until they went to bed. The five-step process began with the interviewer prompting the participant to provide a list of all of the foods consumed. Then, the interviewer guided the respondent through a list of commonly forgotten foods to determine if foods were omitted from the initial list. Details about the timing and characterization of the foods were determined in the third step. During the fourth step, tools were utilized to aid the participant in describing the amount of each food consumed. Finally, the interviewer asked one last time whether anything else was consumed the previous day during the fifth step (144).

Once the AMPM 24-hour dietary recall was complete, NHANES put the data through the Post Interview Processing System (PIPS) in order to prepare and code data. Finally, Survey Net was utilized for further coding and quality checks. Survey Net was also used for nutrient analyses. The combination of AMPM, PIPS, and Survey Net standardized coding and allowed for range checks throughout the process (144). Blanton and colleagues did a validation study of the AMPM and found that the AMPM differed less from Doubly Labeled Water than other commonly used dietary assessment methods (14 day estimated food record, the Block Food Frequency Questionnaire, National Cancer Institute's Diet History Questionnaire) (145). The dietary assessment methods to which the AMPM was compared

in this paper typically underestimated energy intake, and the micronutrient intake estimates from the AMPM were higher than those from the other dietary intake assessments (145).

All moderating variables were obtained using the dietary data collected during the 24-hour dietary recall. Calculations for the aHEI 2010 and the DASH diet adherence score (not provided in the raw NHANES datasets) are described in the *Data Coding* section.

Outcome measures: MetS, CVD and T2DM

MetS is a collection of risk factors for CVD and T2DM. MetS has traditionally been examined dichotomously in adults with the following clinical criteria for diagnosis (based on the National Cholesterol Education Program's Adult Treatment Panel): waist circumference >102 cm (40 inches) for men and >88 cm (35 inches) for women, triglycerides \geq 150 mg/dL, HDL <40 mg/dL for men and <50 mg/dL for women, blood pressure \geq 130 systolic / \geq 85 diastolic mm Hg, and fasting glucose \geq 110 mg/dL (146).

Wijndaele et. al. argued that a continuous MetS score is more valid and should be used for epidemiologic studies (147). Furthermore, because the prevalence of metabolic syndrome is lower in the adolescent population (8.6%) than the adult population (21% to 38.9%) (40), it was prudent to analyze MetS continuously for this study.

Blood pressure and waist circumference were measured in NHANES 2001-2006 during the physical examination, and triglycerides, HDL, and fasting glucose concentrations were obtained through analyses of blood samples. In NHANES 2001-2006, all participants over eight years of age had their blood pressure measured by a physician in the Mobile Examination Center (MEC) (148, 149). Waist circumference was measured on all participants aged two years and older using standardized protocol involving the right ileum as a bony landmark for placement of the tape measure. Measurements were recorded to the

nearest 0.1 cm (150). Fasting glucose and insulin were only measured on participants aged 12 years and older in NHANES whose examination was in the morning (after a nine hour fast). Triglycerides were measured in participants aged 12 and older (151). The continuous MetS risk score was used as the outcome measure of MetS for the adolescents included in this study.

CVD risk was assessed via hypertension and dyslipidemia status. Hypertension was initially characterized ordinally including normal blood pressure, prehypertension (systolic or diastolic BP readings between 90th and 95th percentile for age/height or >120/80 mm Hg), Stage 1 hypertension (systolic or diastolic BP readings (different visits) > 95th percentile for age/height and < 99th percentile + 5 mm Hg), and Stage 2 hypertension (systolic or diastolic BP readings (different visits) >99th percentile for age/height + 5 mm Hg) (38). Dyslipidemia was also initially characterized ordinally including acceptable cholesterol, borderline hypercholesterolemia (TC 170-199; LDL 110-129), and hypercholesterolemia (TC \geq 200; LDL \geq 130) (38). An ordinal variable was generated initially for T2DM including no diabetes, prediabetes (fasting plasma glucose (FPG) 100-125 mg/dL), and diabetes (FPG>125 mg/dL) (152).

Confounding variables

Analyses were controlled for several potential confounders. Analyses were adjusted for age, gender, and socioeconomic status – all traditionally adjusted for in similar studies (108, 110, 116, 118). Socioeconomic status was assessed using Poverty Income Ratio. All analyses were adjusted for race/ethnicity because significant racial differences exist in cotinine concentrations amongst the US population with non-Hispanic Blacks having higher concentrations of cotinine than other races (153, 154). Total energy intake was also

controlled for in analyses when appropriate. It is important to adjust for energy intake because taking in a lot of calories is associated with micronutrient intake but is also associated with obesity which is a direct cause of MetS, CVD, and T2DM.

2.4 ANALYTIC METHODS

Data Coding

In order to minimize risk of misclassification, smokers were excluded from analyses. Self-reported smoking was used in conjunction with cotinine concentration since adolescents may not readily admit that they smoke due to social stigma, fear of their parents finding out, and fear of legal consequences. Sources from the literature were utilized to identify appropriate cut points for distinguishing between smokers and non-smokers. In 1992, Wagenknecht and colleagues published a paper regarding cotinine concentrations in young adults. In the CARDIA study of young adults aged 18-30 years, an overall cutoff of 14 ng/mL was found to have the highest sensitivity and specificity; however, due to racial differences, a cutoff of 9 ng/mL for white participants and 15 ng/mL for Black participants was found to be ideal for distinguishing between smokers and non-smokers (153). However, more recently, Benowitz and colleagues (2009) analyzed NHANES data, arguing that cutoffs may have changed due to changing concentrations of ETS exposure amongst the U.S. population with declines in smoking and indoor-smoking bans (154). Using receiver operator characteristic curve analyses applied to NHANES data, Benowitz et. al. came up with cut points for adults and adolescents to distinguish smokers from non-smokers. The cut points for adolescents (12 to 19 years) varied by race/ethnicity and gender (154). The cut points used for this study (derived from Benowitz et. al. 2009) were: 8.78 ng/mL for non-Hispanic white males, 6.01 ng/mL for non-Hispanic black males, 1.18 ng/mL for Mexican-

American males, 2.95 for non-Hispanic white females, 2.81 ng/mL for non-Hispanic black females, 0.66 for Mexican-American females, and 2.99 ng/mL for other races/ethnicities (154).

The more recent cotinine concentrations described above, in conjunction with self-reported smoking status, were utilized to exclude smokers from the analyses in this study. For those remaining in the dataset, self-report and serum cotinine concentration (analyzed continuously) were utilized to assess level of exposure to ETS.

Though reference ranges were available for some of the mediating variables of interest, all mediating variables (serum α -tocopherol, serum β -carotene, serum folate, red blood cell folate, serum vitamin C) were initially evaluated continuously. The rationale for this decision is that a decrease in any one of these variables may have effects on chronic disease prior to reaching deficiency.

The Alternate Healthy Eating Index score was calculated according to the description by McCullough et. al. 2002 (62). The nine components that make up the aHEI are total fruit, total vegetables (excluding potatoes), cereal fiber, ratio of red to white meat, nuts and soy protein, multivitamin use, percent calories from trans-saturated fat, polyunsaturated to saturated fat ratio, and alcohol. Points are assigned for each component based on whether recommendations were met. For continuous components, intermediate consumption was scored accordingly (i.e., for vegetables, zero servings = zero points, five servings = 10 points, and servings between zero and five will receive between zero and 10 points). The components were scored using data from the 24-hour dietary recall in order to permit calculation of the aHEI score ranging from 2.5 to 87.5 (62).

A DASH diet adherence score was calculated for the adolescents based on guidelines outlined by Mellen et. al. 2008 and Racine et. al. 2011(143, 155). Participants earned a point for every DASH score target they met for a total of up to nine points. An intermediate DASH accordance score was also calculated. Participants earned a full point for meeting the full DASH score target and half a point for meeting the intermediate target. The DASH score targets (and intermediate targets) were <6% (<11%) kilocalories from saturated fat, <27% (<32%) kcals from total fat, >18% (>16.5%) of kcals from protein, <71.4 (<107.1) mg cholesterol/1000 kcals, >14.8 (>9.5) g fiber/1000 kcals, <1143 (<1286) mg sodium/1000 kcals, >238 (>158) mg magnesium/1000 kcals, >590 (>402) mg calcium/1000 kcals, and >2238 (>1534) mg potassium/1000 kcals. Participants were considered DASH accordant if their DASH score was 4.5 or higher and intermediately accordant if their intermediate DASH score was 4.5 or higher (143, 155).

The MetS risk score for children was calculated by summing the age, gender, and race-adjusted z-scores of the following variables: waist circumference, HDL-C (z-score * -1 because higher is better), triglycerides, MAP, HOMA-IR (35, 36). HOMA-IR is a measure of insulin resistance calculated using fasting insulin and fasting glucose. The formula for calculating HOMA-IR is: $[\text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mmol/ml)}] / 22.5$ (156).

Hypertension status and hypercholesterolemia status were derived using the National Cholesterol Education Program (NCEP) Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (157). Participants were classified as non-hypertensive if their blood pressure was below the 90th percentile, pre-hypertensive if either their systolic or diastolic blood pressure was between the 90th and 95th percentiles or their blood pressure was greater than 120/80 mm Hg, Stage 1 hypertension if

their systolic or diastolic was $>95^{\text{th}}$ percentile for age and height but less than the 99^{th} percentile + 5 mm Hg, and Stage 2 hypertension if their systolic or diastolic blood pressure reading was $>99^{\text{th}}$ percentile + 5 mm Hg for age and height. Classifications were based on the average of their blood pressure readings on the day of the exam (157).

Dyslipidemia was also assessed as an ordinal variable including acceptable cholesterol, borderline hypercholesterolemia (TC 170-199; LDL 110-129), and hypercholesterolemia (TC ≥ 200 ; LDL ≥ 130) using cholesterol values provided in the NHANES datasets (39).

T2DM status was also recoded to an ordinal variable (no diabetes, pre-diabetes, and diabetes). Fasting plasma glucose (FPG) 100-125 mg/dL was coded as prediabetes, and FPG >125 mg/dL was coded as diabetes. FPG less than 100 mg/dL was considered “no diabetes” (152).

Exploratory Data Analyses

Graphing procedures (i.e., histograms) and simple statistical analyses (measures of central tendency, variability, and simple regression analyses) were conducted to examine the distribution and relationships between variables. Specifically, distributions were examined for normality and, if not normal, were transformed. Moderator variables and outcome variables which did not already exist in the data were calculated as described above.

Testing Hypotheses

All analyses for this study accounted for the complex survey sampling by using survey weights n_i . Each person i in the NHANES dataset represents n_i persons in the U.S. population. The disease risk variable indicates a probability π_{ij} of person i being diagnosed

with the disease j ; which was interpreted to mean that $y_{ij} = \pi_{ij} * n_{ij}$ of the n_{ij} persons will be diagnosed with disease j .

AIM 1: Examine the relationship between ETS and dietary intake and diet quality in adolescents.

H1: Greater exposure to ETS as measured by self-report and cotinine concentrations will be associated with lower scores on measures of dietary quality (alternate Healthy Eating Index 2010 and DASH diet).

H2: Greater exposure to ETS will be associated with higher intake of saturated fats and lower dietary intake of micronutrients (folate, vitamin C, β -carotene, and α -tocopherol) and fiber.

The hypotheses for aim 1 were tested using multivariable linear regression analyses. The first model constructed only included the two variables of interest (i.e., cotinine concentration and aHEI score). The p-value/confidence interval for the β coefficient was evaluated. Then, potential confounders were added to the model. The β coefficients were examined again for significance to determine whether the coefficient for the effect of the independent variable of interest was affected by the addition of confounding variables into the model.

The following example model demonstrates how one of the hypotheses was tested:
$$E(\pi_{ij} = y_{ij}/n_{ij}) = \beta_0 + (\beta_{1j} * ETS) + (\beta_{2j} \dots \beta_{pj} * \text{control variables}) \dots + \epsilon_{ij}$$
, where π_{ij} is the aHEI score of individual i , the β terms are regression coefficients; β_0 = average aHEI score when cotinine concentration = 0, β_{1j} = the change in average aHEI score per one unit increase in cotinine concentration when other variables in the model are held constant, and ϵ_{ij} = error.

AIM 2: Determine whether nutrient biomarkers mediate the relationship between ETS and chronic disease risk in adolescents.

H1: Greater exposure to ETS will be associated with higher continuous metabolic syndrome score and higher prevalence of hypertension, dyslipidemia, and T2DM.

H2: Greater exposure to ETS will be associated with lower red blood cell folate, serum folate, plasma ascorbic acid, and trans- β -carotene concentrations.

H3: Lower concentrations of red blood cell folate, serum folate, plasma ascorbic acid, and trans- β -carotene will be associated with higher continuous metabolic syndrome score and higher prevalence of hypertension, dyslipidemia, and T2DM.

H4: Part of the relationship between ETS and chronic disease risk (continuous MetS risk score, hypertension, dyslipidemia, and T2DM) will be mediated by red blood cell folate, serum folate, plasma ascorbic acid, and trans- β -carotene concentrations.

AIM 3: Determine whether dietary intake moderates the relationship between ETS and chronic disease risk in adolescents.

H1: Higher scores on measures of quality/patterns of dietary intake (alternate Healthy Eating Index 2010, DASH diet) will attenuate the positive association between ETS exposure and continuous MetS risk score, hypertension, dyslipidemia, and T2DM.

H2: Greater intake of saturated fats and lower dietary intake of micronutrients (folate, vitamin C, vitamin A, and vitamin E) and fiber will exacerbate the positive association between ETS exposure and continuous MetS risk score, hypertension, dyslipidemia, and T2DM.

For analyses using a continuous outcome measure (i.e., MetS risk score), multivariable linear regressions were used. When the outcome was ordinal (i.e., no hypertension, prehypertension, and hypertension), a logit model was used. Finally, when the outcome was dichotomous (i.e., hypercholesterolemia: yes or no), logistic regression analyses were used.

Mediation was tested using the Sobel and Goodman tests in STATA while adjusting for study design (158-160). The procedure involved estimating ab which is the product of the effect of the exposure on the mediator (a) and the effect of the mediator on the outcome (b). The product, ab , was then divided by the square root of the standard error of ab ($s_{ab} = \sqrt{b^2 s_a^2 + a^2 s_b^2 + s_a^2 s_b^2}$) to obtain a test statistic to be compared to the normal distribution (158, 161-163).

To test for interaction, an interaction term was included in the regression analyses. The interaction term was the product of the exposure and the moderator, and the coefficient associated with this product was the “interaction term” (164). The following example model demonstrates how one of the hypotheses related to interaction was tested: $E(\pi_{ij} = y_{ij}/n_{ij}) = \beta_{0j} + (\beta_{1j} * ETS) + (\beta_{2j} * aHEI) + (\beta_{3j} * ETS * aHEI) + (\beta_{4j} \dots \beta_{pj} * \text{confounding variables}) \dots + \epsilon_{ij}$, where π_{ij} is the MetS score of individual i , the β terms are regression coefficients; β_{0j} = average MetS score when all other variables = 0 (i.e., aHEI score = 0, cotinine = 0), β_{1j} = the change in average MetS score per one unit increase in cotinine concentration when other variables in the model are held constant and aHEI = 0, β_{2j} = the change in average MetS score per one unit increase in aHEI score holding all other variables constant and cotinine = 0, β_{3j} = the coefficient for interaction between aHEI score and cotinine concentration, and ϵ_{ij} = error.

If the coefficient β_{3j} is statistically significant, then the relationship between cotinine concentration and MetS score differs based on aHEI score.

Sample size considerations

Because the analyses were secondary data analyses of data that was already collected, the sample size was predetermined. In sample size calculations and power analysis, the variables that influence one another are the size of the sample, the power, and the effect size. Typically, when multiple analyses are being performed, the analysis requiring the largest sample size is the analysis for which necessary sample size is calculated. In this study, the analysis requiring the largest sample size was the test for interaction between two continuous variables (cotinine and aHEI score) in a multiple linear regression with a continuous outcome (continuous MetS score).

Tests of moderation usually have lower effect sizes than other tests with an average effect size of approximately 0.009 (165). When one or both of the variables involved in the interaction is continuous, the power given the same sample size and effect size is even lower (166). The equation used to determine detectable effect size for an interaction in multiple linear regression is:

$$f^2 = \frac{r^2_{Y.MI} - r^2_{Y.M}}{1 - r^2_{Y.MI}} .$$

In the above equation, r^2 represents the amount of variation in the outcome that can be explained by a particular variable or set of variables where $r^2_{Y.MI}$ is the r^2 for the combination of the main effects of the exposure and the moderator separately and the interaction effect and $r^2_{Y.M}$ is the r^2 for the main effects of the exposure and the moderator only. These r^2 values are unknown.

In nutrition, R^2 values are typically low, but can take on a range of values. For example, the R^2 for a model predicting serum cholesterol increased by 0.05 for men and 0.04 for women when dietary and lifestyle variables were added to the model (167). In a study of the effects of intakes of foods from various food groups on mammalian lignan enterolactone, which is protective against hormone-related cancers, a full model including variables representing dietary intake and other covariates (i.e., BMI, smoking), the R^2 for the full models fitted ranged between 0.129 and 0.166 (168). A study examining the effect of saturated fat intake on bone density found R^2 for the entire model to be 0.18-0.59 depending on the measure of bone density taken and other covariates included in the model (169).

Table 2.1 displays simulations of effect sizes given varying estimates of $r^2_{Y.MI}$ and $r^2_{Y.M}$ and the sample sizes required to detect those effect sizes. In the social sciences, an effect size for an interaction term of 0.02 is considered “small”, 0.13 is “moderate”, and 0.35 is considered “large” (170, 171). A sample size of $n=392$ is required to detect an interaction effect size of 0.02, $n=55$ is required to detect f^2 for interaction of 0.13, and $n=26$ is required to detect f^2 for interaction of 0.35 (170, 171).

Table 2.1. Interaction effect sizes given varying R^2 values (171)

$r^2_{Y.MI}$	$r^2_{Y.M}$	f^2	Interaction effect size characterization	Sample size required to detect interaction effect
0.11769	0.10000	0.0200	Small	392
0.21570	0.20000	0.0200		
0.31370	0.30000	0.0200		
0.41179	0.40000	0.0200		
0.50980	0.50000	0.0200		
0.60785	0.60000	0.0200		
0.20357	0.10000	0.1300	Moderate	55
0.29205	0.20000	0.1300		
0.38053	0.30000	0.1300		
0.46901	0.40000	0.1300		
0.55753	0.50000	0.1300		
0.64602	0.60000	0.1300		
0.33331	0.10000	0.3500	Large	26
0.40740	0.20000	0.3500		
0.48149	0.30000	0.3500		
0.55557	0.40000	0.3500		
0.62964	0.50000	0.3500		
0.70371	0.60000	0.3500		

There were 4,692 non-smoking adolescents aged 12 to 19 years with cotinine data in the NHANES 2001-2006 datasets. After applying other exclusion criteria, the smallest sample size for any of the analyses for this study was 1,858. This sample size clearly exceeds the sample size required to detect a small effect size for interaction.

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Chapter 3

AN EXAMINATION OF THE RELATIONSHIP BETWEEN ENVIRONMENTAL TOBACCO SMOKE EXPOSURE AND DIETARY INTAKE AND QUALITY IN ADOLESCENTS.

ABSTRACT

When examined together, health behaviors such as smoking and diet are correlated. The relationship between environmental tobacco smoke (ETS) exposure and diet quality has not been examined in the adolescent population. Data from 4,663 non-smoking, non-pregnant adolescents between 12 and 19 years old who had self-reported (SR) ETS exposure, cotinine, and 24-hour dietary recall data from the National Health and Nutrition Examination Survey (NHANES) years 2001-2006 were utilized to examine whether ETS exposure and dietary quality and intake are related in adolescents. ETS exposure in the home was SR by 15.83% (SE 0.01) of adolescents and 47.86% (SE 0.00) had cotinine \geq 0.05 ng/mL. General linear models were run using the svy command in STATA. SR ETS exposure in the home was related to lower Alternate Healthy Eating Index (AHEI) 2010 score ($\beta = -2.14$, 95% CI -3.68, -0.60) and Dietary Approaches to Stopping Hypertension (DASH) score ($\beta = -0.23$, 95% CI -0.40, -0.05). Cotinine \geq 0.05 ng/mL was also related to lower diet quality scores (AHEI 2010: $\beta = -1.97$, 95% CI -3.05, -0.90; DASH: $\beta = -0.25$, 95% CI -0.39, -0.12). SR ETS exposure and cotinine were related to higher percent calories from saturated fat and lower total fiber, vitamin C, and β -carotene intake. Cotinine was related to lower total folate intake. Neither SR ETS exposure or cotinine was related to α -tocopherol intake. In the United States, adolescents who are

exposed to ETS are more likely to have poor diet quality than unexposed adolescents and may be at greater risk of chronic disease.

3.1 INTRODUCTION

Studies have demonstrated that those who smoke are less likely to be physically active, are more likely to consume alcohol, and have poorer diet quality than those who do not smoke (1, 2). There are many theories behind this clustering of health behaviors ranging from uni-dimensionality (the belief that someone who values health would logically apply that value to multiple health behaviors) to similar environments or characteristics (i.e. socioeconomic status) affecting those health behaviors, to biological influences (1).

Secondhand smoke (called environmental tobacco smoke (ETS)) causes a variety of negative health outcomes including asthma (3, 4), sleep-disordered breathing (5), allergic sensitization (6), cardiovascular disease morbidity and mortality (7-9), lung cancer (10), and stroke (11) and increased risk of smoking (12). A prospective study of a cohort of 37,343 women found an association between ETS exposure and diabetes (13). Clustering of risk factors or experiences could occur for those exposed to secondhand smoke due to familial relationships and shared environments or due to individual-level factors. Since it has been demonstrated that smokers have poorer diets than non-smokers, it is possible that secondhand smoke-exposed individuals may also have poorer diets than those not exposed to secondhand smoke. For example, parents can influence their children's diet quality through their food choices and the creation of a food environment (14-16).

Examinations of the differences in biomarkers of micronutrient concentration between ETS exposed and unexposed individuals have found that ETS-exposed children and adults have lower concentrations of nutrient biomarkers such as vitamins E (17, 18) and C (18-22), folate (18, 23, 24), and β -carotene (18, 19, 25, 26) when compared with unexposed children and adults.

The evidence for differences in dietary intake between ETS-exposed and unexposed individuals is mixed, sparse, and many of the studies are from 10 years ago or more. A few studies conducted in Europe and Asia have found ETS exposed individuals consume fewer vegetables and milk (27, 28), fruit (29), lean meats and complex carbohydrates (27), and beans (28) and more fried food (29). Furthermore, diets of those exposed to ETS have been found to be lower in iron, β -carotene (30), and fiber (27). However, no differences were observed between the diets of non-smokers who live with a smoker when compared to those who live with non-smokers in Japan (31).

ETS-exposed adults in the United States have lower intakes of fruits and vegetables (32-34), consume more fat and cholesterol and less calcium, fiber, and vitamins A and C than non-exposed adults (35, 36).

There are several gaps in the literature regarding differences in dietary intake of ETS exposed and unexposed individuals. The majority of the research in this area has focused on female adults. Studies have not evaluated the differences in diet amongst adolescents by ETS exposure. Furthermore, most studies have utilized self-report to assess ETS exposure rather than an objective measure like cotinine concentration. Finally, the majority of the studies examined intakes of individual foods and nutrients but failed to examine differences in overall diet quality.

The goal of this study is to determine whether the diets of adolescents exposed to ETS differ from the diets of non-exposed or less-exposed adolescents. This study will fill gaps in the literature by examining this relationship in the adolescent population, by using both self-reported and objective (cotinine) measurements of ETS, by examining a large, nationally representative sample of the adolescents in the United States including both males and females, and by exploring not only differences in intake of individual nutrients, but also measures of overall diet quality which are established predictors of chronic disease risk. Hypotheses included that greater exposure to ETS as measured by self-report and cotinine concentrations would be associated with lower scores on measures of dietary quality (AHEI 2010, DASH diet adherence score) and that greater exposure to ETS would be associated with higher intake of saturated fat and lower dietary intake of micronutrients and fiber.

3.2 METHODS

Study Sample

NHANES is an ongoing cross-sectional national survey which collects data from a representative sample of adults and children in the United States. NHANES datasets are available publicly and include variables that can be used to account for the complex sampling design including masked variance pseudo primary sampling unit, masked variance pseudo stratum, and survey weights (37, 38). The survey combines interviews (self-report responses), dietary recall, and physical examinations, and the collection of biologic samples (urine, blood). The analyses for this study were conducted amongst participants 12 to <20 years of age from the National Health and Nutrition Examination

Survey (NHANES) years 2001-2006 who responded to questions about ETS exposure and diet.

Study Variables

The variables utilized in this study are derived from the self-reported responses to surveys in NHANES, data from the 24-hour dietary recall, and results from laboratory tests run on biospecimen samples collected in the Mobile Examination Center (MEC). Consent was obtained from adults and assent was obtained from participants ages seven through 17 years (39).

An individual's ETS exposure was measured in NHANES via self-reported exposure and serum cotinine concentration. These were assessed on all participants ages 12 years and older, however, blood samples would only be taken from those who did not meet any of the exclusion criteria for a blood draw (i.e. hemophilia, chemotherapy within the past four weeks, and medical conditions present on their arms)(40-42). Self-reported tobacco smoke exposure is assessed by several questions in the NHANES questionnaire. For the purposes of this study, the question regarding whether any smokers live in the home was utilized to characterize self-reported ETS exposure. All serum cotinine concentrations were determined using isotope dilution-high performance liquid chromatography / atmospheric pressure chemical ionization tandem mass spectrometry by the Division for Environmental Health Laboratory Sciences at the Centers for Disease Control and Prevention. In NHANES 2001-2002, the limit of detection for cotinine changed from 0.05 to 0.015 (43). The limit was 0.015 for 2003-2006(44, 45). Because the individuals whose samples were analyzed prior to the lower detection limit would not

have had the opportunity to have a valid value below 0.05, for this study, any values below 0.05 were re-coded to the below the detectable limit value.

Dietary intake is assessed in NHANES using a 24-hour dietary recall which utilizes the USDA multiple-pass method (46). The 24-hour dietary recall is conducted in-person during the study examination in the Mobile Exam Center (MEC). The 24-hour dietary recall was completed for all participants in person during their study examination and then a second recall was conducted telephonically by a phone center (47). Only participants for whom 24-hour dietary recall data was deemed “reliable and met the minimum criteria” were included in these analyses (48). For 2001 data collection, a 24-hour dietary recall was considered reliable and meeting minimum criteria if fewer than 25 percent of foods reported lacked descriptions, fewer than 15 percent of reported foods had no quantity, and if every meal reported had a minimum of one identified food (48). For 2002-2006 data, minimum criteria were that the participant completed at least the first four of the five steps of the Automated Multiple Pass Method and that all foods reported for all reported meals had to be identified (48). The NHANES datasets include variables quantifying the amount of saturated fat, fiber, vitamin C, vitamin E, β -carotene, and folate in the items that the participants reported consuming in the past 24-hour period. Data from the 24-hour dietary recall was utilized to generate the diet quality/pattern scores. To do this, the United States Department of Agriculture (USDA) My Pyramid Equivalents Database (MPED) was merged with the NHANES data files (49).

The alternate Healthy Eating Index 2010 (AHEI 2010) score includes nine components: total fruit, total vegetables (excluding potatoes), whole grains, sugar-

sweetened beverages and fruit juice, nuts and legumes, red/processed meat, trans fat, long-chain (n-3) fats (EPA and DHA), polyunsaturated fatty acids, sodium, and alcohol. Protocol for scoring for the AHEI 2010 are described elsewhere (50). Saturated fat intake was examined instead of trans fat intake for the purposes of this study as there is currently no accurate mechanism for calculating trans fat intake using NHANES 2001-2006 data. Reductions in saturated fat consumption have been demonstrated to reduce risk for cardiovascular disease (51). Possible scores for the alternate Healthy Eating Index range from 0 to 110.

Twenty four-hour dietary recall data were also used to calculate the Dietary Approaches to Stop Hypertension (DASH) diet adherence score. The scoring protocol for DASH diet adherence has been described elsewhere (52). Components involved in calculation of DASH diet adherence include percent energy from total and saturated fat and protein, magnesium, calcium, sodium, potassium, and cholesterol consumption per 1,000 calories. The highest possible adherence score is nine.

Covariates in these analyses include age, gender, race/ethnicity, and Poverty Income Ratio (PIR) category (as an estimate of socioeconomic status) – all concepts which have traditionally been adjusted for in similar studies as they are common correlates to health behaviors (1, 2, 27, 30). The demographic portion of the sample person questionnaire administered during the study visit includes questions about ethnicity (if the respondent considers him or herself to be Hispanic) and race. The NHANES dataset contains a variable which summarizes the participants' responses to the race/ethnicity question where the categories are Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, or Other Race – Including Multi-Racial.

Multiple age variables are included in the dataset, but the age in months at the date of the exam was utilized for the purpose of this study. The PIR is the ratio of the total family income to the income demarcating poverty as outlined by the Department of Health and Human Services guidelines in the Federal Register, adjusted for the size of the family, the year, and the state of residence and was used to examine socioeconomic status (53). All distributions were examined for assumptions of normality. Non-normally distributed variables were transformed. If the transformation did not sufficiently normalize the data, then a categorical variable was created and used for analyses. PIR was categorized into three groups: less than 1 (poor), 1 or greater but less than 2 (near poor), and greater than or equal to 2 (not poor).

Sample Size

Data from NHANES 2001-2006 were combined. First, children under 12 years of age and adults 20 years old or older were excluded, leaving 6,861 adolescents. Other exclusion criteria included: positive pregnancy test (n=145), participants whose dietary recall data were not deemed reliable (n=302), missing an answer to the question about whether anyone in the home smoked (n=79), missing serum cotinine (n=658), self-reported smoking in the past 5 days (n=804), and missing self-reported smoking in the past 5 days (n=611). Race- and age-specific cotinine cutoffs derived by Benowitz and colleagues were used to eliminate potential current smokers from the dataset (54). After removing individuals who did not meet the inclusion criteria for all other reasons including self-reported smoking, 342 adolescents were removed from the sample because, despite reporting not smoking, their cotinine value was higher than their age-

/race-specific cutoff. A total of 4,663 adolescents were included in the sample for this study after these exclusions were made.

Statistical Analysis

STATA was used for the statistical analyses for this study. First day dietary recall survey weights provided in the dataset were utilized to calculate a 6-year survey weight according to NHANES protocol (55). For analyses where the svy function was available in STATA (a function which allows for adjustment for complex survey design and weights in statistical analyses), analyses accounted for the 6-year survey weight, masked variance pseudo primary sampling unit, and masked variance pseudo stratum. For analyses where the svy function was not available, the 6-year survey weight was used.

Upon examination, PIR, cotinine concentration, folate intake, vitamin c intake, β -carotene intake, vitamin E intake, and dietary fiber intake were not normally distributed. For all but PIR and cotinine concentration, \log_{10} -transformation resulted in a more normal distribution. Even after multiple types of transformations, serum cotinine was still not normally distributed. Because 52.14 percent of the adolescents in the sample did not have detectable cotinine, for this study, a binary variable was created to distinguish between those who had cotinine values below the detectable limit or <0.05 ng/mL and those who had detectable cotinine values ≥ 0.05 ng/mL. Participants whose cotinine values were above established cut points used to distinguish between adolescent smokers and non-smokers were not included in the sample. These cut points were: 8.78 ng/mL for non-Hispanic white males, 6.01 for non-Hispanic black males, 1.18 for Mexican-American males, 2.95 for non-Hispanic white females, 2.81 for non-Hispanic black

females, and 0.66 for Mexican American females. No specific cutoff was available for other ethnicities, so the general adolescent cutoff of 2.99 ng/mL was used (54).

The response to the question about whether or not a smoker lived in the home was used as a binary measure of self-reported ETS exposure (84.15 percent of the adolescents in the sample reported no smokers living in their home).

To examine the relationship between ETS and dietary intake, general linear models were run. Analyses accounted for probability-based survey strata, clusters, and weights using STATA's SVY command (56). Analyses were first run with just the exposure of interest and the outcome and then potential confounding variables were added into the model. Covariates included in the models were age in years, gender, race/ethnicity, and PIR category.

3.3 RESULTS

Study Sample Characteristics

Table 3.1 presents the characteristics of adolescents in the study sample, adjusted for study design and weights. The average age of the adolescents in the sample was 15.51 years. The majority of the sample was categorized as not poor; however, 18.93 percent were at or below poverty level. The majority of the sample (84.15 percent) reported no smokers in their home. Average cotinine concentration, DASH diet adherence score, aHEI 2010 score, and nutrient intakes are also reported in Table 3.1.

Table 3.2 presents the relationship between self-reported ETS exposure and cotinine. Self-reported ETS exposure was statistically significantly related to cotinine ≥ 0.05 ng/mL (Design-based Pearson $\chi^2 = 830.66$, $p=0.00$) with 51.81% (SE = 2.39) reporting no ETS at home and having cotinine < 0.05 ng/mL and 15.52% (SE = 1.33)

reporting that someone does smoke in the home and having cotinine ≥ 0.05 ng/mL. A larger proportion of those reporting no ETS exposure in the home had cotinine ≥ 0.05 ng/mL (32.36%, SE = 2.09) than those reporting having a smoker in the home with cotinine < 0.05 ng/mL (0.32%, SE = 0.08). This is expected since there are other possible sources of ETS exposure (i.e. neighborhood, peers) and since individuals may not be willing to admit if someone in the home smokes.

General Linear Models

AHEI 2010

The results from the general linear models examining the relationship between self-reported ETS and cotinine and AHEI 2010 score in adolescents are presented in Table 3.3. Self-reported ETS exposure (Model 1) and having a cotinine concentration > 0.05 ng/mL (Model 2) were both statistically significantly related to lower AHEI 2010 scores after adjustment for covariates.

DASH Adherence

The results from the general linear models examining the relationship between self-reported ETS and cotinine and DASH adherence score are presented in Table 3.3. Self-reported ETS exposure (Model 1) and cotinine ≥ 0.05 ng/mL (Model 2) were both statistically significantly related to lower DASH adherence score after controlling for potentially confounding variables.

Nutrient Intakes

Table 3.3 demonstrates the results from the general linear models examining the relationship between binary self-reported ETS, binary cotinine concentration, and nutrient intake in adolescents.

Having a smoker in the home (Table 3.3) was statistically significantly related to a 0.01% higher saturated fat intake when compared to those who did not report having a smoker in the home. Having detectable cotinine ≥ 0.05 ng/mL was also associated with a statistically significant 0.01% higher intake of saturated fat when compared to those with cotinine below 0.05 ng/mL.

Because the micronutrient intakes were not normally distributed amongst this population, nutrient intakes were log-transformed. The coefficients presented in the tables are exponentiated for ease of interpretation. An exponentiated coefficient above 1 indicates that the exposure is associated with greater intake and an exponentiated coefficient below 1 indicates that the exposure is associated with lower intake of the nutrient.

After adjusting for covariates, having a smoker in the home (compared to no smokers in the home) was statistically significantly associated with lower intake of vitamin C (-43%, CI -62%, -14%) β -carotene (-49%, CI -65%, -26%) and fiber (-23%, CI -38%, -5%) when compared to not having a smoker in the home. When compared with cotinine < 0.05 ng/mL or no detectable cotinine, having a cotinine concentration ≥ 0.05 ng/mL was a statistically significant predictor of lower total folate intake (-22%, CI -6%, -35%), vitamin C intake (-49%, CI -22%, -67%), β -carotene (-54%, CI -37%, -66%), and fiber intake (-32%, CI -22%, -42%) even after controlling for covariates.

3.4 DISCUSSION

This is the first study which simultaneously examined the relationship between diet quality, nutrient intakes, and secondhand smoke exposure as measured by self-report and cotinine concentration in a nationally-representative sample of adolescents. Analyses

comparing adolescents based on self-reported ETS exposure and exposure defined by cotinine concentration found that ETS exposure was associated with poorer diet quality as measured by DASH adherence score, AHEI score, lower intake of β -carotene, vitamin C, and fiber, and higher intake of saturated fat. Cotinine concentration ≥ 0.05 ng/mL, but not self-reported ETS exposure, was found to be associated with lower total folate intake.

The findings that ETS exposure was associated with lower intake of fiber (27, 35), β -carotene (27, 30), vitamin C (57), and folate (24) are consistent with results from studies in adult populations. Studies that have examined differences in vitamin E intake between smokers and non-smokers have had mixed results, with some finding no statistically significant difference (58, 59) and others finding that smokers' intake of vitamin E was lower than that of non-smokers (60, 61). In the analyses, there was no significant difference in α -tocopherol intake between ETS-exposed and unexposed adolescents. This is the first study to examine differences in AHEI 2010 score or DASH diet adherence between ETS-exposed and unexposed individuals.

Findings from this study demonstrate that adolescents who are exposed to secondhand tobacco smoke may have poorer diet quality. Clustering of health behaviors can lead to interactions between exposures which affect the same health outcome. This is significant from a public health perspective as it identifies a population with multiple risk factors for chronic disease. Both environmental tobacco smoke exposure and poor diet quality are risk factors for coronary heart disease and diabetes (50, 62-65). This suggests that perhaps interventions targeting ETS-exposed adolescents to reduce exposure should also address improving diet quality in order to maximize benefit for chronic disease risk reduction.

The NHANES dataset is sufficiently large to detect small differences between populations. Furthermore, the population is nationally representative and oversamples minority populations and represents all age groups. However, the cross-sectional nature of this data lends itself to reverse causality in analyses. Therefore, in order to determine directionality of the relationships identified in this study, analyses would need to be replicated with longitudinal data. The sample used for this study excluded adolescents who self-reported smoking and whose cotinine values were above established cut points for suspected active smoking (54). This decreases the chances that smokers were inadvertently included in the analyses, increasing the confidence that the differences are by ETS exposure, not active smoking. However, this also increases the likelihood that those who were heavily exposed to ETS may have been excluded from analyses, which would reduce the strength of the associations observed.

There is likely overlap in the mechanisms by which ETS exposure and poor diet quality increase risk of chronic disease, suggesting the potential for interaction between these exposures and their relationship with chronic disease risk which warrants further investigation.

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Table 3.1 Descriptive characteristics of adolescents from NHANES 2001-2006 adjusted for study design

	Study Sample* N=4,663
Age (months), \bar{x} (SE)	186.09 (0.86)
Sex, % (SE)	
Male	51.13 (0.01)
Female	48.87 (0.01)
Race/Ethnicity, % (SE)	
Mexican American	12.22 (0.01)
Other Hispanic	4.68 (0.01)
Non-Hispanic White	62.61 (0.02)
Non-Hispanic Black	15.96 (0.02)
Other race- including multi-racial	5.52 (0.01)
Poverty Income Ratio category, % (SE)	
>2	61.18 (0.02)
>1 and ≤ 2	19.89 (0.01)
0-1 (at or below poverty level)	18.93 (0.01)
Does anyone in the home smoke?, % (SE)	
No	84.15 (0.01)
Yes	15.83 (0.01)
Cotinine (ng/mL), \bar{x} (SE)*	0.27 (0.02)
Those with cotinine < the highest detectable limit, % (SE)	52.14 (0.03)
Those with cotinine > the highest detectable limit, % (SE)	47.86 (0.00)
DASH diet adherence score, \bar{x} (SE)	2.08 (0.03)
AHEI 2010 score, \bar{x} (SE)	25.78 (0.34)
Saturated fat intake (% of total kcals), \bar{x} (SE)	11.43 (0.00)
Fiber intake (grams per 1000 kcals), \bar{x} (SE)	6.24 (0.07)
Vitamin C intake (mg per 1000 kcals), \bar{x} (SE)	40.12 (1.16)
Folate intake (mg per 1000 kcals), \bar{x} (SE)	188.48 (3.17)
β -carotene intake (mg per 1000 kcals), \bar{x} (SE)	573.76 (26.24)
Vitamin E intake (mg per 1000 kcals), \bar{x} (SE)	2.87 (0.04)

*When using svy command, SE was not estimated due to stratum with single sampling unit. For this variable, obtained SE by running mean with the survey weight only. The means matched using the svy and non-svy commands.

Table 3.2 Relationship between self-reported ETS exposure and cotinine ≥ 0.05 ng/mL

N=4662		Does anyone in the home smoke?	
		No	Yes
Cotinine	<0.05 ng/mL	51.81% (SE 2.39)	0.32% (SE 0.08)
	≥ 0.05 ng/mL	32.36% (SE 2.09)	15.52% (SE 1.33)

Design-based Pearson χ^2 F(1, 45), 830.66 p=0.00

Table 3.3. Self-reported ETS exposure and cotinine as predictors of dietary quality and dietary intake¹

Outcome	Self-reported ETS exposure ²				Cotinine ³			
	n	β	95% CI		n	β	95% CI	
AHEI 2010 score	4480	-2.14**	-3.68	-0.60	4480	-1.97**	-3.05	-0.90
DASH adherence score	4477	-0.23*	-0.40	-0.05	4654	-0.25**	-0.39	-0.12
% kcals from saturated fat	4486	0.01*	0.00	0.01	3048	0.01**	0.00	0.01
Total fiber intake ⁴	4485	0.77*	0.62	0.95	4485	0.68**	0.58	0.78
Total folate intake ⁴	4486	0.87	0.71	1.08	4486	0.78*	0.65	0.94
Vitamin C intake ⁴	4473	0.57**	0.38	0.86	4473	0.51**	0.33	0.78
β -carotene intake ⁴	4475	0.51**	0.35	0.74	4475	0.46**	0.34	0.63
α -tocopherol ⁴	4486	0.90	0.69	1.17	4486	0.86	0.73	1.02

*p<0.05

**p<0.01

¹ Models adjusted for age, gender, race/ethnicity, and PIR category

² Reference group is those with no reported smoker in the home

³ Reference group has no detectable cotinine or cotinine below 0.05 ng/mL

⁴ The outcome variables for these models were not normally distributed and were thus log-transformed. The coefficients for these models were exponentiated for ease of interpretation. A coefficient above 1.0 means the exposure was associated with higher values for the outcome variable and a coefficient below 1.0 means that the exposure was associated with lower values for the outcome variable.

Chapter 4

DO NUTRIENT BIOMARKERS ACT AS MEDIATORS IN THE RELATIONSHIP BETWEEN ENVIRONMENTAL TOBACCO SMOKE AND CHRONIC DISEASE IN ADOLESCENTS?

ABSTRACT

There are known associations between smoking, nutrient biomarkers, and chronic disease in adults; however, the relationship between environmental tobacco smoke (ETS) exposure, nutrient biomarkers, and chronic disease has not been evaluated in adolescents. Data from the National Health and Nutrition Examination Survey (NHANES) years 2001-2006 were pooled to obtain a sample of 3,992 non-smoking, non-pregnant adolescents with blood pressure data and 1,858 adolescents (12-19 years old) with fasting blood sample data. This sample was utilized to determine whether ETS exposure, as characterized by self-report and cotinine concentration, is related to chronic disease and whether this relationship is mediated by nutrient biomarker concentration. The majority of the sample had normal blood pressure (92.1%) and did not have diabetes (86.3%), and 47.2% had normal cholesterol. Linear regressions adjusting for survey design were utilized to examine relationships between ETS exposure and chronic disease. Neither self-reported ETS exposure or cotinine > 0.05 ng/mL were statistically significantly related to diabetes, cholesterol, or blood pressure status. Cotinine > 0.05 ng/mL was positively associated with metabolic syndrome score with and without adjustment for Poverty Income Ratio category. Serum folate, ascorbic acid, and trans- β -carotene were negatively associated with metabolic syndrome score, and red blood cell folate was not statistically significantly associated. Sobel tests were conducted to determine if nutrient

biomarkers mediated the relationship between ETS exposure and metabolic syndrome score and found that serum folate and trans- β -carotene, but not ascorbic acid, were partial mediators of this relationship. This study suggests that ETS exposure is related to metabolic syndrome risk and that this relationship may be mediated by nutrient biomarkers in adolescents. Longitudinal studies are needed to examine these relationships further.

4.1 INTRODUCTION

The link between tobacco smoke and chronic disease risk is well established. Smoking increases the risk of elevated blood pressure and metabolic syndrome in adolescents (1, 2). In adults, smoking is related to greater risk of diabetes (3). Direct smoking is not the only way tobacco smoke can impact chronic disease risk; environmental tobacco smoke (ETS) exposure is associated with greater risk of cardiovascular disease (CVD), type 2 diabetes, and metabolic syndrome in both adults and children (2, 4-7). ETS exposure begins in childhood and adolescence. It is important to better understand the relationship between ETS exposure and risk factors for chronic disease in the pediatric population so that it can be addressed properly.

Exposure to ETS induces oxidative stress, inflammation, platelet aggregation, and endothelial dysfunction – all contributors to chronic disease (8). ETS-exposed individuals appear to have higher concentrations of two biomarkers of CVD risk, fibrinogen and homocysteine, than unexposed individuals (8-10). A growing body of evidence indicates that a possible biological explanation for the relationship between ETS exposure and diabetes/metabolic syndrome is the positive association between ETS exposure and insulin resistance (2, 11).

There is also a demonstrated relationship between ETS exposure and concentrations of nutrient biomarkers. Measures of ETS exposure are associated with lower concentrations of folate, vitamin C, β -carotene, and vitamin A (12). The association between ETS exposure and lower nutrient biomarker concentration has also been found in children (13). Several studies have demonstrated a dose-dependent negative association between ETS exposure and ascorbic acid concentrations in both children and adults (14, 15). Associations between ETS exposure and ascorbic acid concentrations often persist after adjustment for intake (16). There is also ample evidence for lower concentrations of carotenoids in the presence of ETS exposure in both adults and children and adolescents after adjustment for intake (12, 13, 16).

Reduced concentrations of nutrient biomarkers have been associated with chronic disease. There is a biological basis for this relationship; some micronutrients (i.e. retinol, α -carotene, and β -carotene) are required for epithelial cell differentiation and cell signaling and communication. Other micronutrients act as antioxidants which shield cell membranes from damaging effects on lipid peroxidation (13). Folate is critical for the breakdown of homocysteine – an amino acid that has detrimental effects to vasculature (17). Micronutrients in the blood can reduce cell proliferation and protect against platelet adhesion (13). Epithelial cell differentiation, cell signaling and communication, insulin production, oxidation, cell proliferation, and platelet adhesion are all processes which can increase risk of CVD, type 2 diabetes, and metabolic syndrome (18).

As just shown, many of the mechanisms by which low nutrient biomarker concentrations affect chronic disease risk overlap with those proposed for ETS exposure and risk of chronic disease. Therefore, it is possible that the relationship between ETS

exposure and chronic disease is mediated by the impact that ETS exposure has on nutrient biomarker concentration. No research to date has examined this potential mediating relationship. If some of the harmful effects of tobacco smoke are a result of the effect on micronutrient concentrations, then improvements in diet quality may be able to mitigate some of those harmful effects.

This study will examine the effect of ETS exposure on measures of chronic disease risk amongst adolescents and the potential mediating role of micronutrient concentrations in this relationship using a large, nationally representative dataset, the National Health and Nutrition Examination Survey (NHANES).

4.2 METHODS

Study Sample

NHANES is a cross-sectional national surveillance survey conducted continuously across the United States with a complex sampling design including masked variance pseudo primary sampling unit, masked variance pseudo stratum, and survey weights (19, 20). The survey involves a combination of self-reported interviews, dietary recall, and physical examinations including collection of biologic samples (urine, blood). The analyses for this study were conducted amongst participants 12 to 19 years of age from the National Health and Nutrition Examination Survey (NHANES) years 2001-2006 who answered questions about ETS exposure and diet and who underwent a physical examination including blood samples. Consent was obtained from participants who were 18 years old and older and from guardians of participants under age 18, and assent was provided by participants under 18 years of age (21).

Study Variables

NHANES involves an interview conducted in participants' homes and an examination by interviewers and medical professionals in a Mobile Examination Center (MEC) (22). This study examined data related to ETS exposure, nutrient biomarkers, and chronic disease risk. One of the ETS exposure variables and the demographic variables were obtained via self-report during surveys. Laboratory test results from biospecimen sample data collected during the exam in the MEC were utilized for one of the other measures of ETS exposure, for the nutrient biomarker concentrations, and for some of the measures of chronic disease risk. The MEC examination also included anthropometric and blood pressure measurements which were utilized for this study as part of the metabolic syndrome score. The adolescents under 18 years of age in this sample provided assent and an adult provided consent; adolescents 18 years old or older provided consent (21).

The NHANES survey includes a demographics portion which includes questions about race/ethnicity, household income, age, gender, and other variables. Some of this information was utilized to derive the metabolic syndrome score and some were utilized as covariates in analyses. The first question about race/ethnicity determines whether or not the participant considers him/herself to be Hispanic. The next question asks about race. These questions are combined into a race/ethnicity variable with the following categories: Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic, or Other Race – Including Multi-Racial. While the NHANES dataset has several variables for age, the age in months on the day of the MEC examination was utilized for this study.

Poverty income ratio (PIR) is a variable included in the NHANES dataset as a measure of socioeconomic status. This variable is a ratio of the family income reported during the survey to an income specific to the family size, geographic location, and year (determined by the Federal Register) (23). PIR is not normally distributed, so for the purposes of this study, a categorical variable was created to represent poor ($PIR < 1$), near poor ($1 \leq PIR < 2$), or not poor ($PIR \geq 2$) (23).

The NHANES questionnaire includes multiple questions to assess self-reported ETS exposure. The question utilized for this study read, “I would now like to ask you a few questions about smoking. Does anyone who lives here smoke cigarettes, cigars, or pipes anywhere inside this home?” A self-reported variable from the MEC examination about tobacco/nicotine use in the past five days was also utilized to eliminate smokers from the dataset.

Anthropometric measurements are taken during the MEC exam. For this study, waist circumference measurements were utilized to generate the metabolic syndrome score. Exams were taken with the participant wearing an examination gown. In order to standardize waist circumference measurements, NHANES examiners palpate the hip to locate a bony landmark on the ilium (where it intersects with the midaxillary line of the body) and mark it with a pencil, always taking the measurement from the right side of the participant. A steel measuring tape is then placed around the participant and mirrors are utilized to ensure that the tape measure is level. Examiners are careful to ensure that the tape measure is snug without compressing the skin. Measurements are taken at the end of a normal breath to the nearest millimeter (24).

Blood pressure measurements are also taken during the MEC exam. Participants are asked to sit quietly for five minutes prior to blood pressure measurements. A MEC examiner uses a mercury sphygmomanometer to take three to four blood pressure measurements using a standardized procedure (25). For this study, the first blood pressure measurement was utilized.

Blood draws in NHANES are taken from participants three years old and older but are restricted to those who did not meet any of the exclusion criteria for a blood draw (i.e. medical conditions present on their arms, hemophilia, or chemotherapy within the past four weeks). Self-reported environmental tobacco smoke exposure and cotinine concentration are assessed on all participants ages 12 years and older. NHANES methodologies used to determine cotinine and blood cholesterol (26-35) and procedures for measurement of blood pressure (23, 28, 36) are described elsewhere. Briefly, CDC's Division for Environmental Health Laboratory Sciences determines cotinine concentration via isotope dilution-high performance liquid chromatography / atmospheric pressure chemical ionization tandem mass spectrometry. Data from NHANES 2001-2006 was utilized for this study, and during that time period, the limit of detection changed from 0.05 ng/mL to 0.015 ng/mL (26). Due to this change, any value below 0.05 ng/mL was recoded to be below the detectable limit since individuals with cotinine <0.05 ng/mL whose lab tests were performed prior to this change would not have had detectable cotinine.

For analyses involving fasting blood samples (i.e. fasting plasma glucose, insulin, cholesterol), the fasting subsample 2-year MEC weights were utilized to calculate a 6-year fasting subsample weight used for adjustment in analyses. For all other analyses, the

2-year dietary weights were used to create a 6-year dietary weight for use in analyses.

The public datasets include variables for these design characteristics which can be used to control for sampling design in analyses (20). Data from the self-reported responses to surveys in NHANES, the 24-hour dietary recall, the physical examination, and results from laboratory tests run on bio-specimen samples collected in the Mobile Examination Center (MEC) were used to derive variables for these analyses.

A continuous metabolic syndrome score was also calculated by summing the age, gender, and race-adjusted z-scores for waist circumference, mean arterial pressure, homeostasis model assessment, HDL-cholesterol, and triglycerides (37, 38).

Because research in Chapter 3 demonstrated that ETS-exposed adolescents have different dietary intake and quality when compared to ETS-unexposed adolescents, dietary intake variables were also generated using data from the 24-hour dietary recall administered as a part of NHANES in the MEC. The 24-hour dietary recall used in NHANES utilizes the multiple pass method developed by the USDA (39). Included in the NHANES dataset is summary nutrient information indicating, from the foods and beverages reported, how much vitamins A, C, E, and folate was consumed in the preceding 24-hours (40). For an approximate measure of “adequacy” of intake of the micronutrient of interest, the participants’ intake was compared to the Recommended Dietary Allowance (RDA) from the National Academy of Sciences’ Dietary Reference Intakes based on the age and gender of that participant. Dichotomous variables were created to represent whether or not the participant met the RDA for each nutrient being examined (41). The RDA is an amount set for each nutrient which is believed to meet the

needs of approximately 98 percent of the population (42). These variables were utilized as covariates in the mediation analyses.

Variable distributions were evaluated for normality. Any non-normally distributed variables were log-transformed. If log-transformation did not normalize the variable, other transformations were attempted, and for those which could not be normalized, a dichotomous or categorical variable was created.

Sample Size

After combining data from NHANES 2001-2006 and eliminating adults (20 years old and older) and children (less than 12 years of age), 6,861 adolescents remained in the dataset. Individuals were excluded if they had a positive pregnancy test (n=145), a dietary recall data deemed “not reliable” (n=302), missing self-reported ETS exposure in the home (n=79), missing serum cotinine (n=658), if they reported smoking in the past 5 days (n=804), or if there was no data for self-reported smoking in the past 5 days (n=611). Potential smokers were eliminated from the dataset (1,783 adolescents) based on age-/race-specific cotinine cutoffs (43). Of the 4,663 adolescents remaining, for analyses where metabolic syndrome score, cholesterol, or diabetes were the outcomes, additional exclusions were applied for those adolescents missing total cholesterol (n=20), LDL cholesterol (n=2,503), triglyceride (n=2,442), HDL-cholesterol (n=20), blood pressure (n=671), or a 2-year fasting subsample weight (n=2427), leaving 1,858 adolescents in the sample. For this paper, this sample is referred to as the “fasting sample”. For analyses where blood pressure was the outcome variable, only those missing blood pressure were excluded, leaving a sample of 3,992 adolescents in the “blood pressure sample”.

Statistical Analysis

Statistical analyses were conducted using STATA. Distributions of variables were examined to determine if they were normally distributed. The variables were examined separately for both samples (fasting sample and blood pressure sample). Metabolic syndrome score, red blood cell folate, serum folate, and serum ascorbic acid were all normally distributed for both samples. Several variables were transformed or categorized due to a non-normal distribution. Poverty income ratio was categorized into less than 1 (poor), 1 or greater but less than 2 (near poor), and greater than or equal to 2 (not poor). In both the blood pressure sample and the fasting sample, the proportion of adolescents with cotinine < 0.05 ng/mL was just over 50 percent. As a result, cotinine was converted to a binary variable where those with non-detectable cotinine or cotinine under 0.05 ng/mL had a value of 0 and those with cotinine ≥ 0.05 ng/mL had a value of 1.

Mediation analyses were performed in Stata using the `sgmediation` command to run Sobel-Goodman tests with the `svy` prefix to account for survey design (44). If a represents the coefficient when the mediating variable is regressed on the independent variable and b represents the relationship when the dependent variable is regressed on the mediating variable, the Sobel test is the ratio of the product of ab to the estimate of the standard error of ab (45, 46).

4.3 RESULTS

Study Sample Characteristics

Descriptive statistics for the fasting study sample and the blood pressure sample are presented in Table 4.1. The mean age of the adolescents in the fasting sample was 186.41 months (15.53 years; SE = 0.94 months) and in the blood pressure sample was

185.94 months (15.50 years; SE = 0.89 months). Both samples were roughly half male, half female with slightly more males than females. Over 60 percent of both samples had a Poverty Income Ratio (PIR) greater than 2 (2 times the poverty level) and under 20 percent of both samples had a PIR at or below poverty level. Only 14.17 percent (SE = 0.02) of the fasting sample and 15.96 percent (SE = 0.01) of the blood pressure sample reported that anyone in the home smoked. Just under half of each sample had cotinine that was greater than or equal to 0.05 ng/mL.

Table 4.2 presents descriptive statistics for the measures of chronic disease risk and concentrations of micronutrients. The mean continuous metabolic syndrome score was -0.14 (SE = 0.13) in the fasting study sample. As expected, the majority of the sample had normal blood pressure (92.05%) and 5.38% were pre-hypertensive, 2.10% were Stage 1 Hypertensive, and 0.47% had Stage 2 hypertension. Based on total cholesterol, LDL-cholesterol, and triglyceride, 47.23% of the sample had normal cholesterol, 30.79% had borderline high cholesterol and 21.98% had high cholesterol. Less than one percent of the sample had diabetes (0.52%), 13.22% had pre-diabetes, and 86.26% did not have diabetes.

Environmental Tobacco Smoke Exposure and Chronic Disease Risk

Prior to examining the potential mediating effects of concentrations of nutrients in the relationship between environmental tobacco smoke and chronic disease risk, analyses were conducted to verify that relationships between ETS and measures of chronic disease did exist amongst this population. For ETS and continuous metabolic syndrome score, linear regressions were conducted adjusting for survey design with and without controlling for poverty. These analyses did not control for age, race/ethnicity, or gender

because those are all factors used to generate the metabolic syndrome score. Self-reported ETS was not statistically significantly related to continuous metabolic syndrome score; however, cotinine ≥ 0.05 ng/mL was associated with higher metabolic syndrome score before ($\beta = 0.31$, 95% CI 0.05, 0.58) and after ($\beta = 0.29$, 95% CI 0.01, 0.56) adjustment for poverty income ratio category (results not shown).

Because such a small proportion of the population had diabetes and the proportion of the population with pre-hypertension and Stage 1 and Stage 2 hypertension was also extremely low, diabetes and blood pressure status were dichotomized into no diabetes vs. pre-diabetes/diabetes and normal blood pressure vs. pre-hypertension/hypertension (stages 1 and 2).

Self-reported ETS exposure was not statistically significantly related to blood pressure (OR=0.88, CI 0.48, 1.60) even after adjustment for race/ethnicity and PIR category (OR=0.92, CI 0.50, 1.70). Cotinine was also not statistically significantly associated with blood pressure (OR=1.12, CI 0.78, 1.62) after adjustment for race/ethnicity and PIR category (OR=1.12, CI 0.82, 1.80). Gender and age were not included in these models since these are used directly in determining blood pressure status.

Self-reported ETS exposure was not found to be associated with diabetes (OR=0.65, CI 0.39, 1.08) even after adjustment for sex, age, race, and PIR category (OR=0.67, CI 0.40, 1.12). Cotinine greater than or equal to 0.05 ng/mL was also not statistically significantly related to diabetes (OR=1.32, CI 0.96, 1.84) after adjustment for the same covariates (OR=1.46, CI 0.98, 2.17).

Self-reported ETS exposure was not found to be associated with borderline or high cholesterol (OR=1.16, CI 0.91, 1.46) even after adjustment for sex, age, race/ethnicity, and PIR category (OR=1.18, CI 0.92, 1.50). Cotinine ≥ 0.05 ng/mL was also not associated with cholesterol (OR=1.16, CI 0.84, 1.62) after adjustment for the same covariates (OR=1.07, CI 0.74, 1.53).

Nutrient Concentration and Chronic Disease Measures

Before analyzing whether or not nutrient biomarker concentrations mediate the relationship between ETS exposure and metabolic syndrome, analyses were conducted to verify an association between nutrient concentrations and metabolic syndrome. Linear regression analyses were conducted with and without covariates, controlling for study design, to determine whether red blood cell folate, serum folate, ascorbic acid, or trans- β -carotene concentration were statistically significantly related to metabolic syndrome score. Red blood cell folate was not statistically significantly associated with metabolic syndrome score even after adjustment for PIR category. Increased serum folate was associated with lower metabolic syndrome score ($\beta = -0.06$, CI -0.09, -0.03) even after adjustment for PIR category ($\beta = -0.06$, CI -0.08, -0.03). Higher ascorbic acid concentration was also associated with lower metabolic syndrome score ($\beta = -0.80$, CI -1.37, -0.23) after adjustment for PIR category ($\beta = -0.81$, CI -1.38, -0.24). Similarly, higher trans- β -carotene was associated with lower metabolic syndrome score ($\beta = -1.12$, CI -1.41, -0.84) after adjustment for PIR category ($\beta = -1.13$, CI -1.42, -0.84).

Mediation

Table 4.3 presents the results from the mediation analyses. Since serum folate, ascorbic acid, and trans- β -carotene were the only nutrient concentrations statistically

significantly related to metabolic syndrome score, these variables were examined as potential mediators in the relationship between cotinine and metabolic syndrome. Just over 30 percent (30.2%) of the total effect of cotinine on metabolic syndrome score was mediated by serum folate concentration. The coefficient for the Sobel test was 0.1 and the p-value was <0.01 . The relationship between cotinine and metabolic syndrome score was also mediated by trans- β -carotene with 67.2 percent of the total effect being mediated, a Sobel coefficient of 0.2, and a p-value <0.01 . The Sobel coefficients remained statistically significant even after adjustment for PIR and meeting the RDA for folate and vitamin A intake respectively (see Table 4.3). The Sobel coefficient for analyses examining whether serum ascorbic acid mediates the relationship between ETS and metabolic syndrome was not statistically significant (Sobel coefficient of 0.05, $p>0.05$) with or without adjustment for PIR and meeting the RDA for vitamin C.

4.4 DISCUSSION

This study sought to evaluate whether the impact of ETS exposure on chronic disease risk is mediated by micronutrient concentration. Analyses did not identify a statistically significant relationship between self-reported ETS exposure and chronic disease measures. The only measure of chronic disease found to be associated with cotinine was continuous metabolic syndrome score. Examination of the relationship between micronutrient concentration and metabolic syndrome score revealed that serum folate, ascorbic acid, and trans- β -carotene were all inversely related to metabolic syndrome score. Subsequent investigation into potential mediating relationships found that the relationship between cotinine and metabolic syndrome score was partially mediated by serum folate, ascorbic acid, and trans- β -carotene.

The lack of association between ETS exposure and blood pressure, cholesterol, and diabetes in this study does not necessarily rule out the possibility that ETS exposure can increase risk for these conditions. These conditions all have relatively low prevalence in the adolescent population as compared to the adult population since they develop over time. Other studies which have examined the relationship between ETS exposure and blood pressure in adolescents have also found that while it appeared that ETS exposure was related to increased blood pressure, the relationship did not reach statistical significance (1). It is possible that the effect of the ETS on chronic disease takes time to manifest itself. A study of French women found higher incidence of type 2 diabetes amongst women who reported exposure to ETS during childhood (47). The exposure amongst the women in that study was also greater than the exposure in this sample with 58 percent of the sample in the Lajous study reporting exposure to ETS during childhood as compared to under 20 percent of this sample reporting that someone in the home smokes.

While the blood pressure, cholesterol, and diabetes variables in this study were dichotomous, the metabolic syndrome score utilized in this study is a continuous measure of chronic disease risk taking into account subclinical risks related to blood pressure, insulin resistance, and cholesterol and therefore would allow for detection of associations that may be missed when examining clinical disease cutoffs.

Weitzman and colleagues used a dichotomous characterization of metabolic syndrome and found that ETS exposure resulted in a 4.7-fold increase in prevalence of metabolic syndrome (CI, 1.7 to 12.9) when compared to ETS-unexposed adolescents as characterized by cotinine concentration (2). This study characterized metabolic

syndrome continuously, but also found that cotinine >0.05 ng/mL was associated with increased metabolic risk.

Wilson and colleagues found that vitamin C, vitamin E, cis- β -carotene, trans- β -carotene, and folate were all lower with increasing exposure to ETS (12). This supports the argument that part of the relationship between ETS exposure and chronic disease risk may be mediated by decreases in micronutrient concentration.

There is evidence for the biological plausibility of a mediating relationship between ETS exposure, micronutrient concentration, and chronic disease risk. In vitro, free radicals contained in tobacco smoke reduce antioxidant concentration in plasma (48). Depleted vitamin C concentration is associated with inflammation, atherosclerosis, and peripheral arterial disease (49). In analysis of NHANES data, Ford and colleagues discovered an association between c-reactive protein (a marker of inflammation) and lower concentration of vitamin C and β -carotene, amongst other micronutrients (50). Ford and colleagues also found in yet a different study of adult NHANES participants that metabolic syndrome was associated with lower concentrations of carotenoids and vitamin C (50). As Roberts and Sindhu explained in their mini review of oxidative stress and metabolic syndrome, oxidative stress plays a key role in the development of hypertension, diabetes, and atherosclerosis (18). Therefore, it is logical that depletion of nutrients which function as antioxidants would be associated with increased risk of these diseases.

NHANES is a large, nationally representative dataset. This study investigated relationships between ETS exposure and chronic disease using two different characterizations of ETS exposure – self-report and cotinine concentration. Cotinine

concentration is a more objective measurement of ETS exposure than self-report. Yet another strength of this study is the use of both clinical cutoffs/definitions of chronic disease like hypertension, diabetes, and hypercholesterolemia as well as a subclinical indicator of chronic disease risk – continuous metabolic syndrome score. The use of the continuous metabolic syndrome score in this study is especially important given that the population of interest was adolescents. ETS exposure may have a gradual effect on chronic disease risk over time which may not present as clinical disease until adolescents reach adulthood. However, by examining continuous metabolic syndrome score, this study was able to examine whether there were trends for harmful effects of ETS exposure on chronic disease risk before development of full blown disease, and whether these harmful effects were mediated by nutrient concentration.

The cross-sectional nature of the NHANES data poses the potential for identification of relationships which are not causal in nature due to the lack of temporality. Future studies which first characterize ETS exposure and later assess nutrient concentration and chronic disease risk would provide stronger evidence for causality in the mediation relationship observed here.

The potential mediating relationship between ETS exposure, nutrient concentrations, and chronic disease suggests that these relationships should be evaluated further using longitudinal data. More importantly, if part of the harmful effect of ETS exposure on chronic disease risk is mediated by nutrient concentrations, this suggests the possibility that a diet with adequate or higher micronutrients (specifically folate, ascorbic acid, and trans- β -carotene) could mitigate these harmful effects. Further research should be conducted to determine whether increased intake of these micronutrients and a

healthier diet overall can reduce the harmful effects of ETS exposure on chronic disease risk.

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Table 4.1 Descriptive characteristics of adolescents from NHANES 2001-2006 adjusted for study design

	Fasting study sample* N=1,858	Blood pressure sample* N=3,992
Age (months), \bar{x} (SE)	186.41 (0.94)	185.94 (0.89)
Sex, % (SE)		
Male	50.35 (0.02)	51.38 (0.01)
Female	49.65 (0.02)	48.62 (0.01)
Race/Ethnicity, % (SE)		
Mexican American	12.01 (0.01)	12.26 (0.01)
Other Hispanic	3.72 (0.01)	4.24 (0.01)
Non-Hispanic White	62.64 (0.02)	63.73 (0.02)
Non-Hispanic Black	14.93 (0.02)	14.31 (0.02)
Other race- including multi-racial	6.70 (0.01)	5.47 (0.01)
Poverty Income Ratio category, % (SE)		
PIR greater than 2 (2*poverty level)	61.53 (0.02)	61.29 (0.02)
PIR greater than poverty level (1) but less than or equal to 2 (2*poverty level)	21.60 (0.01)	19.90 (0.01)
PIR 0 to 1 at or below poverty level	16.87 (0.01)	18.81 (0.01)
Does anyone in the home smoke?, % (SE)		
No	85.83 (0.02)	84.04 (0.01)
Yes	14.17 (0.02)	15.96 (0.01)
Cotinine (ng/mL), % (SE)		
<0.05 ng/mL	50.88 (0.03)	51.92 (0.02)
\geq 0.05 ng/mL	49.12 (0.03)	48.10 (0.02)

*Blood pressure sample = eligible adolescents with cotinine and self-reported ETS data, valid 24-hour dietary recall data, and blood pressure values; Fasting study subsample = eligible adolescents from the blood pressure sample who also had fasting plasma blood glucose and cholesterol values

Table 4.2 Descriptive characteristics of adolescents from NHANES 2001-2006 adjusted for study design

	Fasting study sample* N=1,858	Blood pressure sample* N=3,992
Continuous MetS risk score, \bar{x} (SE)	-0.14 (0.13)	-
Blood pressure status, % (SE)**	-	-
Normal blood pressure	-	92.05 (0.01)
Pre-hypertension	-	5.38 (0.01)
Stage I Hypertension	-	2.10 (0.00)
Stage II Hypertension	-	0.47 (0.00)
Cholesterol status, % (SE)**		-
Normal cholesterol	47.23 (0.02)	-
Borderline hypercholesterolemia	30.79 (0.02)	-
Hypercholesterolemia	21.98 (0.02)	-
HDL status, % (SE)**	-	-
Normal	67.28 (0.02)	-
Borderline low	17.93 (0.01)	-
Low	14.79 (0.01)	-
Diabetes status, % (SE)**	-	-
No diabetes	86.26 (0.01)	-
Pre-diabetes	13.22 (0.01)	-
Diabetes	0.52 (0.00)	-
Red blood cell folate, \bar{x} (SE)	247.45 (4.16)	251.73 (3.72)
Serum folate, \bar{x} (SE)	12.63 (0.24)	13.15 (0.19)
Ascorbic acid, \bar{x} (SE)	1.01 (0.02)	1.06 (0.02)
Trans- β -carotene, \bar{x} (SE)	12.30 (0.35)	12.16 (0.21)

*Fasting study sample = eligible adolescents with cotinine, self-reported ETS data, valid 24-hour dietary recall data, and fasting blood draw data; Blood pressure sample = eligible adolescents with cotinine, self-reported ETS data, valid 24-hour dietary recall data, and blood pressure data

**Blood pressure, cholesterol, and diabetes status based on values from one study visit.

Table 4.3. Sobel test for mediation relationship between ETS, nutrient biomarkers, and MetS

Mediator	Sobel coefficient	Standard error	P-value	Percent of total effect that is mediated
Folate	0.1	0.0	0.00*	30.2
Folate ¹	0.1	0.0	0.02*	28.8
Ascorbic acid	0.1	0.0	0.10	15.2
Ascorbic acid ²	0.1	0.0	0.10	16.3
Trans- β -carotene	0.2	0.1	0.00*	67.2
Trans- β -carotene ³	0.2	0.1	0.00*	65.9

*Statistically significant at the 0.05 level

¹Adjusted for PIR and whether or not participant met the RDA for folate

²Adjusted for PIR and whether or not participant met the RDA for vitamin C

³Adjusted for PIR and whether or not participant met the RDA for vitamin A

Chapter 5

DO MEASURES OF DIET QUALITY OR DIETARY INTAKE MODERATE THE RELATIONSHIP BETWEEN ENVIRONMENTAL TOBACCO SMOKE EXPOSURE AND METABOLIC SYNDROME RISK IN ADOLESCENTS?

ABSTRACT

Both dietary intake and environmental tobacco smoke (ETS) exposure affect chronic disease risk. Using data from the 2001-2006 National Health and Nutrition Examination Surveys, this study examined whether there is an interaction between diet quality/intake and ETS exposure in their relationship with chronic disease risk in non-smoking, non-pregnant adolescents ages 12-19 years. Data analyses were performed using STATA. General linear models adjusting for study design were utilized to examine potential interactions between ETS exposure, as characterized by self-report and cotinine concentration, and dietary quality (as characterized by alternate Healthy Eating Index score and Dietary Approaches to Stopping Hypertension score) and intake (fiber, vitamins A, C, and E, and folate). While meeting the Recommended Dietary Allowance (RDA) for dietary folate equivalents (DFEs) was negatively associated with metabolic syndrome score, none of the interactions tested were statistically significant even after adjustment for poverty income ratio (PIR) category and supplement use.

5.1 INTRODUCTION

Dietary intake and environmental tobacco smoke (ETS) exposures affect risk of chronic diseases including metabolic syndrome. Findings in Chapter 3 demonstrated that adolescents exposed to ETS also have diets of poorer quality than those who are unexposed. These adolescents are likely to be at increased risk for chronic diseases. This

study also found that ETS exposure is associated with higher metabolic syndrome score in adolescents; this is consistent with findings in the literature demonstrating that ETS contributes to insulin resistance, hypercholesterolemia, endothelial dysfunction, and increased coagulation, and is associated with metabolic syndrome in adolescents (1). Research in Chapter 4 showed that the relationship between ETS and metabolic syndrome was partially mediated by serum folate, ascorbic acid, and trans- β -carotene concentrations which is supported by other studies in the literature (2). Since part of the effect of ETS exposure on metabolic syndrome was related to nutrient biomarkers, it is possible that diet quality may impact the effect of ETS exposure on metabolic syndrome.

Folate is required for the breakdown of homocysteine (3). Elevated homocysteine concentration is thought to impact chronic disease by promoting blood clot formation, inflammation, creating oxidative stress, causing smooth muscle cells to multiply rapidly, and affecting vasodilation and constriction in the endothelium (4). Variations in antioxidant concentrations also impact chronic disease risk. Oxidative stress results from an imbalance between the oxidative stress and antioxidant capacity in the body. Research indicates that oxidative stress is associated with insulin resistance, adiposity, total body fat, waist circumference, endothelial dysfunction, lipoprotein atherogenicity, and arterial blood pressure – all components of metabolic syndrome (5). Conversely, micronutrient concentration can reduce cell proliferation and protect against platelet adhesion (6). Individuals with chronic metabolic diseases have lower concentrations of vitamin C, carotenoids, and α -tocopherol (5, 7, 8).

Dietary intake can also influence metabolic syndrome risk. The Dietary Approaches to Stopping Hypertension (DASH) diet has been recommended by the

American Heart Association due to observed positive impacts on blood pressure, oxidative stress, inflammation, insulin sensitivity, and cholesterol concentrations (9). The Alternate Healthy Eating Index has been associated with significantly lower risk of chronic disease – specifically coronary heart disease and diabetes (10). Fiber, vitamin C, and vitamin E intake are inversely related to metabolic syndrome in adolescents (11, 12). Antioxidant supplementation has been associated with improvement in flow-mediated dilation of the brachial artery, reductions in hypertension, arterial stiffness, and insulin resistance (5). Some animal, in vitro, and human research supports the potential for dietary intake – specifically fiber and antioxidants - to modify the effect of ETS exposure on chronic disease risk (13-15); however, evidence for interaction between these exposures is mixed (16).

The goal of the present study is to test the potential interaction between diet quality and intake and ETS exposure as they relate to metabolic syndrome score using a large, nationally representative sample of adolescents from the National Health and Nutrition Examination Survey (NHANES). The measures of diet quality to be examined as potential effect modifiers in this study include Dietary Approaches to Stopping Hypertension (DASH) adherence score, the Alternate Healthy Eating Index (AHEI) 2010 score, and whether or not individuals consumed recommended amounts of fiber, vitamin C, folate (dietary folate equivalents), β -carotene (retinol activity equivalents), and vitamin E for their age/gender.

5.2 METHODS

Study Sample

The National Health and Nutrition Examination Survey (NHANES) is a nationwide survey conducted in the United States which gathers health and nutrition data on a representative sample of adults and children through interviews, examinations, dietary recall, and biospecimen collection. A complex survey design is utilized in order to ensure that sufficient participants from minority groups are included to allow for sub-analysis. The publicly available datasets include masked variance pseudo primary sampling unit, masked variance pseudo stratum, and survey weights which can be utilized to adjust for the complex survey design in analyses (17, 18). This study utilized NHANES 2001-2006 data and included participants ages 12 to <20 years who had ETS exposure, dietary recall, and fasting biospecimen data available.

Study Variables

The diet quality and intake variables utilized in this study were derived from the 24-hour dietary recall data. Metabolic syndrome score was calculated using variables from the biospecimen samples and anthropometric measurement data collected in the Mobile Examination Center (MEC). The ETS exposure variable is also from blood samples collected in the MEC. Adolescents 18 years old or older provided consent; adolescents younger than 18 years of age provided assent and their parents provided consent for participation (19). Blood samples were obtained from participants ages 12 years and older who did not have medical conditions on their arms, had not had chemotherapy in the past four weeks, and did not have hemophilia (20-22).

ETS exposure was quantified for this study using the serum cotinine concentration values. The Division for Environmental Health Laboratory Sciences at the Centers for Disease Control and Prevention (CDC) utilized isotope dilution-high

performance liquid chromatography / atmospheric pressure chemical ionization tandem mass spectrometry to assess serum cotinine. The limit of detection changed during collection years 2001-2002 from 0.05 ng/mL to 0.015 ng/mL and remained at 0.015 ng/mL from 2003-2006 (23-25).

A well-tested 24-hour dietary recall method called the USDA automated multiple-pass method (AMPM) was utilized to collect dietary intake data for NHANES during the exam in the MEC and then again by phone by a phone center (26). Participants whose dietary recall data was not deemed “reliable and met the minimum criteria” were excluded from these analyses. In 2001, in order to be considered reliable and meet minimum criteria, descriptions needed to be provided for 75 percent or more of foods reported, quantities needed to be provided for 85 percent or more of reported foods, and meals had to include at least one identified food (27). In 2002-2006, four of the five AMPM steps had to be completed and all foods for all meals needed to be identified (27). The NHANES datasets include summary variables indicating the amount of individual nutrients in all of the foods the participants reported consuming in the 24-hour period along with whether or not the participant reported taking any dietary supplements in the past 30 days. Twenty-four hour recall data from NHANES and United States Department of Agriculture (USDA) My Pyramid Equivalents Database (MPED) data were utilized to create the diet quality/pattern scores (28).

The NHANES dataset contains variables indicating participants’ intake of fiber, dietary folate equivalents (DFE), retinol activity equivalents (RAE), and vitamins C and E based on the foods they reported during their 24-hour dietary recall. The National Academy of Sciences published Dietary Reference Intakes (DRI) indicating

recommendations for intake of various nutrients based on individuals' age and gender (29). These DRIs are based on available data and informed decisions. The goal is not only to prevent disease, but to also achieve optimum health (29). The DRIs include Estimate Average Requirements (EAR), Recommended Dietary Allowances (RDAs), and Adequate Intakes (AIs). The EAR is the amount of a nutrient thought to meet the needs of 50 percent of the population. RDAs are the EAR plus two standard deviations and are thought to meet approximately 98 percent of the population's nutritional needs (30). When insufficient data exist to designate a RDA for a nutrient, an AI is set which is thought to meet the requirements of most individuals of that age and gender (30).

Rather than assess nutrient intakes continuously, the variables in the dataset indicating intake of DFEs, RAEs, vitamins C and E, and fiber were compared to the DRIs and variables were created to indicate whether or not the RDA (for DFEs, RAEs, vitamins C and E) and AI (for fiber) were met (29). The variables were not normally distributed. Furthermore, it is possible that consuming adequate amounts of these nutrients would be the important factor in these relationships and that the relationships may not be continuous.

The alternate Healthy Eating Index 2010 score was developed specifically to be predictive of chronic disease risk by encompassing components already known to be associated with chronic disease risk such as fruit and vegetable, whole grain, nuts and legumes, red/processed meat, trans fat, long-chain (n-3) fats, polyunsaturated fatty acids, sugar-sweetened beverages and fruit juice, sodium, and alcohol consumption. One substitution was made from the scoring protocol described by Chiuve et. al. – saturated fat was included in place of trans fat because NHANES 2001-2006 does not include

information about trans-fatty acid intake (10). Saturated fat intake is also related to chronic disease risk, so this substitution is appropriate for the purposes of this study (31). Alternate Healthy Eating Index scores can range from 0 to 110.

Scoring protocols for the Dietary Approaches to Stopping Hypertension (DASH) diet adherence score have been described elsewhere (32). Twenty four hour dietary recall data from NHANES were utilized to develop this score which draws on the following intake components: calcium, magnesium, potassium, sodium, and cholesterol per 1,000 calories and percent of calories from saturated and total fat and protein. The highest possible score for DASH adherence is nine.

Continuous metabolic syndrome score was calculated for the sample of 1,889 non-smoking (by self-report and cotinine values), non-pregnant adolescents between 12 and 19 years of age with reliable dietary recall data, questionnaire data on ETS exposure, cotinine data, and blood pressure, waist circumference, glucose, insulin, triglyceride, and HDL-cholesterol data. The score was calculated per guidelines presented by Eisenmann et. al. (33). First, blood pressure values were utilized to calculate mean arterial pressure (MAP) and glucose and insulin values were utilized to calculate homeostasis model assessment (HOMA). Then, waist circumference, MAP, HOMA, HDL-cholesterol, and triglycerides were all regressed onto age, race, and sex. The age utilized was the age in months at examination. Gender and race/ethnicity were obtained via the demographic questionnaire. Race/ethnicity is categorized into the following groups: Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic, or Other Race – Including Multi-Racial. The HDL-cholesterol scores were multiplied by negative one

because higher HDL is beneficial for disease risk. Finally, the standardized scores were summed to create the continuous metabolic syndrome score (33).

Sample Size

Adolescents ages 12-19 years (n=6,861) included in the NHANES 2001-2006 datasets were considered for this study. Adolescents were excluded from the study if they were pregnant (n=145), if they did not have reliable dietary recall data (n=302), if they did not have a serum cotinine value (n=658), if they reported smoking in the past 5 days (n=804), and if their value was missing for self-reported smoking in the past 5 days (n=611). Smokers were also removed from the dataset using race/age-specific cotinine ng/mL cut point values (non-Hispanic white males = 8.78; non-Hispanic black males = 6.01; Mexican-American males = 1.18; non-Hispanic white females = 2.95; non-Hispanic black females = 2.81; Mexican American females = 0.66; other ethnicities = 2.99) established by Benowitz and colleagues (n = 1,783) (34). Of the 4,663 adolescents not excluded by the criteria already listed, additional adolescents were removed if they were missing cholesterol, blood pressure, or 2-year fasting subsample weight (total cholesterol = 20, LDL = 2,503, triglyceride = 2,442, HDL-cholesterol = 20, blood pressure = 671, 2-year fasting subsample weight = 242). After these exclusions, 1,858 adolescents remained in the sample.

Statistical Analysis

Statistical analyses for this study were conducted using STATA. Two-year fasting survey weights were utilized to calculate a 6-year survey weight according to NHANES protocol (35). The svy function was utilized for analyses. This command allows for adjustment for complex survey design in analyses. The svyset command was

used to input the 6-year survey weight, masked variance pseudo primary sampling unit, and masked variance pseudo stratum.

All variables were examined to determine if they were normally distributed. If they were not normally distributed, variables were transformed. If a variable's distribution remained non-normal after transformation, a dichotomous or categorical variable was created and utilized for analyses. Several variables were not normally distributed upon examination. Possible values for PIR category included poor ($\text{PIR} < 1$), near poor ($1 \leq \text{PIR} < 2$), and not poor ($\text{PIR} \geq 2$). A large proportion of adolescents had cotinine values at or below the limit of detection. Therefore, for this study, analyses utilized a dichotomous variable for cotinine where those with cotinine < 0.05 ng/mL had a value of 0 and those with cotinine ≥ 0.05 ng/mL having a value of 1.

Analyses did not control for age, gender, or race/ethnicity as these were all utilized to calculate the continuous metabolic syndrome score; however, analyses did include Poverty Income Ratio (PIR) category as a covariate to control for potential confounding due to socioeconomic status. PIR is based on income determined by the Department of Health and Human Services guidelines in the Federal Register to represent poverty. It accounts for family size, location, and year (36).

To examine the potential interaction between ETS exposure and dietary patterns or intake as they relate to metabolic syndrome, general linear models and ordinal regressions with interaction terms were run using the svy command (37). Analyses were first run with ETS exposure, the dietary variable of interest, the interaction term, and the outcome. Potential confounding variables (i.e. PIR category and calories) were added into the model in subsequent steps of the analyses.

5.3 RESULTS

Study Sample Characteristics

Descriptive statistics for the study sample are presented in Table 5.1. The average age of the adolescents was 15.5 years (SE = 0.9 months). Roughly half (50.4 percent) of the sample was male and half (49.7 percent) was female. More than half (61.5 percent) of the sample had a PIR two times the poverty level and only 16.9 percent had a PIR at or below poverty level. Just under half of the study population (49.1 percent) had cotinine ≥ 0.05 ng/mL. Diet quality score and dietary intake score averages and standard errors are also presented in Table 5.1. Less than 10 percent of the study population met the AI for fiber and the RDA for vitamin E. Just over one quarter of the study population (26.0 percent) reported taking a dietary supplement in the past 30 days. The average continuous metabolic syndrome score was -0.1 (SE = 0.1).

General Linear Models

Appendix 5.A. presents results from general linear models testing the relationship between dietary intake and metabolic syndrome score. Log of fiber intake and meeting the RDA for folate were the only variables statistically significantly related to lower metabolic syndrome score. Results from the general linear models examining potential interactions between the effects of diet intake and quality variables and cotinine concentration on continuous metabolic syndrome score are presented in Tables 5.2 and 5.3. DASH diet score, AHEI 2010 score, meeting AI for fiber intake, and meeting the RDA for vitamin C, vitamin E, and RAE intake were not statistically significantly related to metabolic syndrome score and the interaction terms in these models were not statistically significant, with or without adjustment for poverty income ratio.

Meeting the RDA for DFE was statistically significantly associated with lower metabolic syndrome score ($\beta = -0.9$, 95% CI -1.5, -0.4) even after adjustment for total energy intake and PIR category ($\beta = -0.9$, 95% CI -1.4, -0.3). However, the interaction term in these models was not statistically significant ($\beta = 0.7$, 95% CI -0.2, 1.6) even after adjustment for total energy intake and PIR category ($\beta = 0.7$, 95% CI -0.2, 1.6).

Because 26 percent of the population reported using dietary supplements in the past 30 days, analyses were re-run controlling for dietary supplement use. Interaction terms in these models were not statistically significant.

5.4 DISCUSSION

This study sought to examine whether measures of diet quality and dietary intake mitigate the harmful effects of ETS exposure on metabolic syndrome in adolescents. Previous findings in Chapter 4 indicated that part of the relationship between ETS exposure and increased metabolic syndrome score was mediated by biomarkers of folate, trans- β -carotene, and ascorbic acid. Therefore, it was hypothesized that dietary intake may modify the relationship between ETS exposure and metabolic syndrome score. However, analyses did not demonstrate an interaction between ETS exposure and aHEI score, DASH diet score, or measures of dietary intake in their effect on metabolic syndrome.

This study has many strengths such as the nationally representative sample, the standardized study procedures utilized in NHANES, and the use of cotinine as an objective measure of ETS exposure. This study included examination of adequacy of intake of individual micronutrients using RDAs in addition to summary scores of diet quality (AHEI/DASH adherence). While the analyses looking at whether or not the RDA

for individual micronutrients were met on that given day could be chance findings, the diet quality scores are likely to capture diet quality as a whole since they take into account multiple components of dietary intake.

Study limitations include the cross-sectional nature of the study design which excludes the ability to assess temporality and the use of data from just one 24-hour dietary recall.

It is important to consider the relationship between dietary intake and nutrient biomarkers. Dietary intake and nutrient biomarkers are not perfectly correlated. A review of studies of folate intake and biomarkers of folate in the blood found moderate correlations between folate intake as assessed by 24-hour dietary recall and biomarkers of folate (ranging from 0.18 to 0.55) when accounting for supplement use (38). A meta-analysis of studies of dietary intake in adults found that vitamin C intake assessed by dietary recall was positively correlated with plasma vitamin C concentration ($r = 0.46$) and this included studies which accounted for supplement use (39). In one study of adults, the correlation between 24-hour dietary recall, energy- and serum cholesterol-adjusted alpha-tocopherol intake and serum alpha-tocopherol ranged from 0.50 to 0.61 and for carotene ranged from 0.47 to 0.55 (40).

The authors are not aware of systematic evaluations of the correlation between dietary intake and nutrient biomarkers in the adolescent population. Appendix 5.B. presents the correlations between dietary intake and nutrient biomarkers in the adolescent population examined in this study. These analyses were performed in STATA using the CORR_SVY to adjust for survey strata, PSUs, and weights. All of the dietary intake variables examined were statistically significantly correlated to the biomarkers being

examined at the 0.01 level. Analyses were re-run with the dietary intake variable examined per 1,000 kcals, and correlations remained statistically significant. Correlation coefficients were moderate and ranged from 0.2-0.4.

Other studies have not examined these exact interactions, but a recent study examined the impact of meeting the adequate intake for tocopherol on the impact of ETS exposure on lung cancer risk and did find that it had a protective effect (41).

Some older studies demonstrated that antioxidant supplementation can reduce oxidative stress and even mitigate some of the harmful effects of ETS exposure (42-44). However, reviews of the literature have demonstrated that antioxidant supplements do not offer the benefits originally hoped for – instead, they either have no impact or can potentially increase mortality (45). The authors of the review hypothesized that the reason for this potential harmful effect is that there is a delicate balance that may vary from person to person between antioxidants and free radicals and that some free radicals are needed in the body (45). Another theory is that endogenous antioxidants have more of an impact on chronic disease risk than dietary antioxidants (45). If there is, in fact, a narrow range of quantity of dietary antioxidant intake that would be able to affect the relationship between ETS exposure and chronic disease risk, it is possible that the intake of antioxidants amongst this study sample was not within that range.

Another potential explanation for the discrepancy between other study findings and findings from this study is the cross sectional nature of the NHANES. The 24-hour dietary recall data from this study was obtained at the same time point as the ETS exposure variable and the variables used to calculate the metabolic syndrome score. To examine whether diet quality or intake could modify the harmful effects of ETS exposure

an ideal study would be longitudinal in nature. Researchers would need to take baseline measurements of chronic disease risk, assess (and potentially manipulate) diet quality/intake and ETS exposure multiple times, and include follow-up measurement of chronic disease risk. A longitudinal design would not only establish temporality but could also allow time for the effect of the diet quality/intake to become significant enough to be measured.

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Table 5.1 Descriptive characteristics of adolescents from NHANES 2001-2006 adjusted for study design

Age (months), \bar{x} (SE)	186.4 (0.9)
Sex, % (SE)	
Male	50.4 (0.0)
Female	49.7 (0.0)
Race/Ethnicity, % (SE)	
Mexican American	12.0 (0.0)
Other Hispanic	3.7 (0.0)
Non-Hispanic White	62.6 (0.0)
Non-Hispanic Black	14.9 (0.0)
Other race- including multi-racial	6.7 (0.0)
Poverty Income Ratio category, % (SE)	
PIR greater than 2 (2*poverty level)	61.5 (0.0)
PIR greater than poverty level (1) but less than or equal to 2 (2*poverty level)	21.6 (0.0)
PIR 0 to 1 at or below poverty level	16.8 (0.0)
Cotinine (ng/mL), % (SE)	
<0.05 ng/mL	50.9 (0.0)
\geq 0.05 ng/mL	49.1 (0.0)
DASH diet adherence score, \bar{x} (SE)	1.2 (0.0)
AHEI 2010 score, \bar{x} (SE)	25.5 (0.4)
Met Adequate Intake for Fiber, % (SE)	4.8 (0.0)
Met RDA for Vitamin C, % (SE)	44.2 (0.0)
Met RDA for Dietary Folate Equivalents, % (SE)	66.5 (0.0)
Met RDA for Retinol Activity Equivalents, % (SE)	26.7 (0.0)
Met RDA for Vitamin E, % (SE)	6.3 (0.0)
Took dietary supplement in past 30 days, % (SE)	26.0 (0.0)
Continuous MetS risk score, \bar{x} (SE)	-0.1 (0.1)

Table 5.2. Interaction between effects of ETS exposure and DASH diet/AHEI 2010 score adherence on continuous metabolic syndrome score

	DASH adherence			AHEI 2010		
	β	95% CI		β	95% CI	
Unadjusted models (n=1,765)						
Cotinine >0.05 ng/mL ¹	0.2	-0.3	0.7	-0.4	-1.4	0.5
DASH score	0.1	-0.1	0.2			
AHEI 2010 Score				-0.0	-0.0	0.0
Interaction	0.1	-0.2	0.5	0.0	-0.0	0.1
Adjusted models ² (n=1,693)						
Cotinine >0.05 ng/mL ¹	0.2	-0.4	0.7	-0.5	-1.5	0.5
DASH score	0.1	-0.1	0.2			
AHEI 2010 Score				-0.0	-0.1	0.0
Interaction	0.1	-0.2	0.5	0.0	0.0	0.1

*p<0.05

**p<0.01

¹ Reference group has no detectable cotinine or cotinine below 0.05 ng/mL

² Models adjusted for Poverty Income Ratio

Table 5.3. Interaction between effects of ETS exposure and nutrient intake on continuous metabolic syndrome score

		Fiber			Folate			Vitamin C			Vitamin A			Vitamin E		
		β	95% CI		β	95% CI		β	95% CI		β	95% CI		β	95% CI	
Unadjusted models (n=1765)																
Cotinine ¹		0.3*	0.0	0.6	-0.2	-0.9	0.5	0.2	-0.2	0.7	0.3	-0.1	0.6	0.3*	0.1	0.6
Met AI?	Fiber	-0.2	-1.0	0.6												
Met RDA?																
	Folate				-0.9**	-1.5	-0.4									
	Vitamin C							0.0	-0.4	0.4						
	Vitamin A										-0.1	-0.5	0.4			
	Vitamin E													0.0	-0.9	0.8
Interaction term ³		0.5	-0.8	1.8	0.7	-0.2	1.6	0.2	-0.5	0.8	0.1	-0.8	1.1	-0.3	-2.3	1.7
Adjusted models ² (n=1693)																
Cotinine ¹		0.3	-0.0	0.5	-0.3	-1.0	0.4	0.3	-0.2	0.7	0.3	-0.1	0.6	0.3*	0.0	0.6
Met AI?	Fiber	0.0	-0.9	0.9												
Met RDA?																
	Folate				-0.9**	-1.4	-0.3									
	Vitamin C							0.1	-0.4	0.6						
	Vitamin A										0.1	-0.4	0.6			
	Vitamin E													0.2	-0.7	1.1
Interaction term ³		0.6	-0.6	1.8	0.7	-0.2	1.6	0.1	-0.6	0.7	0.1	-0.9	1.1	-0.3	-2.3	1.7

*p<0.05

**p<0.01

¹ Reference group has no detectable cotinine or cotinine below 0.05 ng/mL

² Models adjusted for total caloric intake and Poverty Income Ratio

³ Interaction term is between the variable indicating whether or not recommended intake for the nutrient was met and the cotinine variable

Appendix 5.A. Dietary intake and continuous metabolic syndrome score estimated using general linear models¹

Independent Variable	Unadjusted model			Adjusted model ²		
	n	β	95% Confidence Interval	n	β	95% Confidence Interval
DASH	1765	0.1	-0.0 0.3	1693	0.1	-0.1 0.3
AHEI 2010 ³	1765	-0.0	-0.5 0.6	1693	-0.0	-0.0 0.0
Percent kcal from saturated fat	1765	-4.4	-8.9 0.1	1693	-3.8	-8.5 0.8
Log fiber intake ³	1765	-0.4**	-0.7 -0.1	1693	-0.4*	-0.7 -0.0
Log vitamin C intake ³	1760	-0.0	-0.2 0.1	1688	-0.0	-0.2 0.2
Log folate intake ³	1765	-0.4*	-0.7 -0.1	1693	-0.3*	-0.7 -0.0
Log beta carot ³	1759	-0.1	-0.2 0.1	1687	-0.0	-0.2 0.1
Log vitamin E ³	1765	-0.2	-0.5 0.1	1693	-0.0	-0.5 0.4
Met folate RDA ³	1765	-0.6**	-0.9 -0.2	1693	-0.5**	-0.9 -0.2
Met fiber RDA ³	1765	-0.1	-0.7 0.6	1693	-0.2	-0.6 0.9
Met vitamin C RDA ³	1765	0.1	-0.2 0.4	1693	0.1	-0.2 0.5
Met vitamin A RDA ³	1765	-0.1	-0.4 0.3	1693	0.1	-0.2 0.4
Met vitamin E RDA ³	1765	-0.2	-1.0 0.7	1693	0.1	-0.9 1.0

*p<0.05

**p<0.01

¹ The outcome variable for all of the models was continuous metabolic syndrome score.

² All adjusted models included PIR category and whether or not the participant reported taking supplements as covariates.

³ Adjusted model also included total caloric intake as a covariate.

Appendix 5.B. Correlations between dietary intake and biomarkers in adolescent sample*

Dietary intake variable	Biomarker	n	Correlation coefficient	P-value
Log of dietary folate equivalents	Serum folate	1779	0.2	0.0
Log of dietary folate equivalents	RBC folate	1774	0.2	0.0
Log of vitamin C intake	Ascorbic acid	1136	0.4	0.0
Log of RAE	Log of trans- β -carotene	1766	0.2	0.0
Log of dietary folate equivalents per 1,000 kcal	Serum folate	1779	0.3	0.0
Log of dietary folate equivalents per 1,000 kcal	RBC folate	1774	0.2	0.0
Log of vitamin C intake per 1,000 kcal	Ascorbic acid	1136	0.4	0.0
Log of RAE per 1,000 kcal	Log of trans- β -carotene	1776	0.2	0.0

*Analyses adjusted for survey strata, PSUs, and weights

Chapter 6

DISCUSSION

While smoking has declined over recent decades and anti-smoking campaigns and legislation have been put into place, smoking is still a problem in the United States.

Smoking is a direct contributor to a plethora of health problems including cardiovascular disease, diabetes, and metabolic syndrome. These same diseases are impacted by exposure to ETS. Adolescents are particularly susceptible to smoking and ETS due to increasing importance of peer influence, incomplete/developing cognitive ability to think abstractly, and the desire to establish autonomy. While adolescent smoking has declined, it is still prevalent in the United States. The same characteristics of adolescence that make adolescents vulnerable to smoking also put adolescents at risk for poor dietary intake. Adolescents in the United States do not consume recommended amounts of fruits and vegetables, and vegetable intake has declined in recent years. Poor diet and diet quality also contribute to chronic disease risk. Furthermore, both diet and ETS exposure affect nutrient biomarkers which are also related to chronic disease risk. Because ETS exposure and poor diet quality can both have a negative impact on chronic disease risk and are both health behaviors of particular concern in adolescence, it is important to study these relationships together.

The goal of this body of work was to expand understanding of the relationship between ETS exposure, dietary quality and intake, nutrient biomarkers, and chronic disease risk in adolescents. This research was designed to identify potential high risk populations in need of targeted intervention to lower chronic disease risk, gain

understanding of how ETS impacts chronic disease risk, and determine whether diet can affect the relationship between ETS exposure and chronic disease risk.

Paper 1 describes analyses that were conducted to examine whether dietary quality and intake differed by ETS-exposure in adolescents to understand whether ETS exposure and poor diet quality coexist in the adolescent population. This important question could identify a higher risk group of adolescents with multiple exposures known to increase risk of chronic disease. The next paper aimed to determine which chronic disease measures were impacted by ETS and explore whether nutrient biomarkers are mediators in this relationship. Understanding how ETS impacts chronic disease is essential for identifying appropriate interventions to mitigate this risk. In the final paper, potential interactions between ETS exposure and dietary quality/intake in their relationship with chronic disease risk were tested. The following sections provide a discussion of the study findings, strengths, limitations, implications for future research, and broader public health impact.

6.1 SUMMARY OF MAJOR FINDINGS

The results of the first paper indicate that ETS exposure was associated with poorer diet quality and intake in adolescents. ETS exposure was associated with lower DASH adherence, AHEI score, β -carotene, vitamin C, and fiber intake, and greater intake of saturated fat in adolescents – all indicative of poorer diet quality. Furthermore, the fact that these associations were statistically significant with or without adjustment for confounding variables and when characterizing ETS-exposure using self-reported ETS exposure and cotinine concentration strengthens the confidence in these findings. Cotinine concentration was also associated with lower total folate intake. The findings

from this study regarding ETS exposure and micronutrient consumption are consistent with the adult literature. This was the first study to examine differences in diet quality as measured by AHEI 2010 and DASH scores by ETS exposure.

ETS exposure increases risk for chronic diseases. If ETS-exposed adolescents are also consuming lower amounts of micronutrients that are protective against chronic disease, this could mean that they are at an even greater risk of development of chronic disease. The combination of ETS exposure and poor diet quality, both with respect to micronutrient consumption and overall diet quality, indicates that these adolescents should be targeted for interventions to address these exposures.

The second paper examined relationships between ETS exposure and chronic disease, nutrient biomarker concentration and chronic disease, and potential mediating relationships. Self-reported ETS-exposure was not statistically significantly related to any of the measures of chronic disease or chronic disease risk. This is not consistent with some studies available in the literature. Under-reporting of ETS exposure could mask a legitimate relationship, so analyses were also conducted with cotinine concentration – a more objective measure of ETS exposure. While not related to hypertension or diabetes, cotinine concentration over 0.05 ng/mL was statistically significantly related to continuous metabolic syndrome score. This is consistent with findings from studies that have examined the relationship between ETS exposure and dichotomously characterized metabolic syndrome. Given that the prevalence of hypertension and diabetes is low in the adolescent population, but the metabolic syndrome score is a continuous measure of disease risk, it would be more difficult to detect a relationship between ETS-exposure and hypertension. Higher serum folate, ascorbic acid, and trans- β -carotene concentration

were each associated with lower metabolic syndrome score. These nutrient biomarkers were also found to partially mediate the relationship between cotinine concentration and metabolic syndrome score. This mediation relationship has not been studied previously.

ETS exposure causes oxidative stress, platelet aggregation, endothelial dysfunction, and inflammation (1). As mentioned in Chapter 2, β -carotene is needed for epithelial cell differentiation, signaling, and communication – all of which can impact chronic disease risk (2). β -carotene is also an antioxidant. It is possible that the oxidative stress, platelet aggregation, inflammation, and endothelial dysfunction caused by ETS (1, 3) is so great that it stresses the body, resulting in lower concentrations of antioxidants in the blood and increased risk of chronic disease.

Finally, the third paper aimed to test the hypothesis that dietary intake/quality modifies the relationship between ETS exposure and chronic disease risk. No statistically significant interactions were identified between DASH diet score, aHEI score, and intake of retinol activity equivalents (RAEs – the unit of measure for vitamin A requirements that adjusts for the differences in bioavailability of various forms of vitamin A), vitamin E, vitamin C, DFEs, or fiber. This is the first study which examined the potential interaction between ETS exposure and dietary intake/quality in the relationship with chronic disease risk in adolescents. While older studies in adults initially found a protective effect of antioxidant supplements against the negative impact of ETS on chronic disease risk, more recent evaluations of the overall impact of antioxidant supplementation on chronic disease risk has demonstrated no protective effect and even potential increases in mortality (4). It is possible that there were not enough

ETS-exposed individuals with higher diet quality scores and greater intake of vitamins A, C, E, folate, and fiber in this sample to observe a moderating relationship.

6.2 STRENGTHS AND LIMITATIONS

All research has both strengths and limitations. This study utilized data from the National Health and Nutrition Examination Survey (NHANES) which is a large, nationally representative survey with strict data protocols and quality control checks integrated throughout. This is a major strength over previous studies of similar topics which have smaller, less diverse samples.

Examination of the relationship between ETS, diet quality, nutrient biomarkers, and chronic disease risk in an adolescent population is also a strength of this study as most studies examine these relationships in adults. Adolescents have greater exposure to ETS than adults. Furthermore, while chronic disease starts to develop in childhood, because fewer adolescents have heart disease, diabetes, and metabolic syndrome than adults, this life stage is an important one for prevention.

The elimination of smokers from the dataset is both a strength and a weakness. The mechanism by which smokers were eliminated is a strength over previous studies as both self-report and previously established cutoff values were utilized to remove smokers. Taking smokers out of the sample also allowed analyses to pinpoint the impact of *environmental* tobacco smoke and not smoking. However, because smokers are likely to have the highest ETS exposure levels, it could also have reduced the power to detect meaningful differences or dampened the magnitude of the effects observed.

Other studies examining chronic disease risk in children often characterize metabolic syndrome dichotomously; however, the prevalence of metabolic syndrome is

not very high in adolescents. Because the variables used to diagnose metabolic syndrome are continuous, a continuous metabolic syndrome score was constructed. Utilizing a continuous metabolic syndrome score is a unique strength of this study. This allows pre-clinical elevations in blood pressure, fasting plasma glucose, triglycerides, waist circumference, and reductions in high density lipoprotein (HDL) cholesterol to be detected and reduces the sample size required to detect relationships.

The main limitation of this study is that the data are cross-sectional and were not designed for evaluating this type of longitudinal relationship. Temporality is one of the criteria for establishing causal relationships; therefore, the findings from this study only suggest plausible relationships which need to be investigated further in a longitudinal or experimental setting. This is especially true for the mediation analyses. Maxwell and Cole demonstrated that using cross-sectional data to test for mediation results in biased estimates even if the mediation relationship is already complete in both autoregressive and random effects change models (5).

Another limitation of this study is that the dietary intake variables were based on one 24-hour dietary recall. Though the use of the Food Frequency Questionnaire (FFQ) data was considered, the NHANES FFQ does not record portion size; therefore, it would not provide an accurate portrait of individuals' food consumption. The 24-hour dietary recall only characterizes the previous day of intake of individuals in the study. One day does not accurately represent any one individual's usual dietary intake. The 24-hour dietary recall data is prone to recall bias; however, the threat of recall bias is reduced for the 24-hour dietary recall by the use of AMPM methodology.

Diagnosis of cardiovascular disease and/or diabetes is often associated with recommendations from clinicians for changes in diet; therefore, reverse causality is a distinct possibility. However, given that the study population was adolescents, reverse causality is less likely than it would be in an adult population. Another limitation of the study was the lack of confirmation of elevated fasting plasma glucose on a second day and high blood pressure on a second day to meet the requirements for diagnosis of diabetes and hypertension (6, 7).

One criticism of the Sobel test is the assumption of a normal sampling distribution for ab when it ordinarily is asymmetrical (8). However, the sampling distribution of ab is usually normal in large samples, so this is less of a concern in this study (9).

Despite the limitations of this study, the research provides important contributions. Novel findings include the discovery that ETS-exposed adolescents have poorer diet quality than their unexposed peers, that ETS-exposure is related to chronic disease risk in adolescents, and that the relationship between ETS-exposure and chronic disease risk may be partially mediated by nutrient biomarkers. Not only does this work provide a greater understanding of the relationship between ETS exposure, diet quality and intake, nutrient biomarkers, and chronic disease risk in adolescents – it also lays the framework for future research and policies.

6.3 IMPLICATIONS FOR RESEARCH AND POLICY

Cardiovascular disease, diabetes, and metabolic syndrome are major public health issues in the United States. In Chapter 2, a study was presented in which the prevalence of metabolic syndrome was reported to be 8.6 percent in adolescents (10). This study examined NHANES data from 2001-2006. A more recent study that examined NHANES

data from 2001-2010 found metabolic syndrome prevalence to be 10.1 percent amongst adolescents (11). Thus, the public health problem still exists and may be on the rise.

Adolescence is an important time for health behavior interventions, especially for smoking and substance abuse-related behaviors and dietary intake. As mentioned in Chapter 2, adolescence is a period when children are establishing their sense of self and autonomy from their parents and when peers become more influential. The ability to think abstractly and understand longer term consequences of behavior is something that develops during adolescence and is not fully developed until adulthood; therefore, adolescents may not be able to ascribe possible negative health impacts to their own health behavior choices. Adolescence is a period of feeling invincible – like nothing bad can happen; this aspect of this developmental life stage could make adolescents more prone to subjecting themselves to ETS exposure and eating unhealthy diets. Finally, health behaviors have been demonstrated to track from adolescence to adulthood, so the formation of healthy behaviors in adolescence is critical.

Because adolescents who are exposed to ETS also have poorer diet, it is likely that they are at an even greater risk for chronic disease because both poor diet quality and ETS exposure are risk factors for chronic disease. The identification of this high risk population suggests that adolescents exposed to ETS may be in need of multiple interventions to decrease their disease risk – both to reduce ETS exposure and improve diet quality.

Future research could determine why ETS exposure and poor diet quality and intake coexist; for example, is this due to familial, peer, or individual influences? Because findings from studies in the literature demonstrate that adults who smoke have

poorer diet quality, it is possible that adolescents with parents who smoke have greater ETS exposure and poorer diets due to the food environment (both food choices and foods that are available) their parents have created. However, because adolescence is a time when children begin to focus more on their peers, peer influences should also be considered. It is possible that adolescents gain exposure to ETS through spending time with peers who smoke and that those peers guide their food choices as well. Identifying the root cause of this problem could be key for finding appropriate interventions to target adolescents.

Chapter 3 demonstrated that over 30 percent of adolescents who reported no ETS exposure in the home had cotinine concentrations ≥ 0.05 ng/mL. NHANES should continue to measure cotinine amongst adolescents because participants may not be forthcoming about their exposure due to the stigma associated with smoking. Furthermore, self-reported exposure questions provide a context for where the exposure occurred.

The relationship between active smoking and chronic disease has been examined, but fewer studies have examined the relationship between ETS exposure and chronic disease, especially in the adolescent population. ETS exposure has been demonstrated to be related to dichotomous metabolic syndrome score; however, this study demonstrated that it is also positively correlated with a continuous metabolic syndrome score, reinforcing the finding that the effects of secondhand smoke on chronic disease can be observed as early as adolescence. This clearly provides further support for tobacco regulations which would limit exposure to adolescents and children; however, it also raises the question of what the longer-term effects of this exposure would be on chronic

disease risk. This finding could be utilized to motivate parents to quit smoking. It also has policy implications. While landlords are allowed to ban smoking from their properties, it is not a requirement. Future legislation could incorporate regulations regarding smoking in rental housing – for example, buildings with over a certain number of units could not allow smoking.

These analyses were conducted with data from 2001-2006. Newer NHANES data is available and these analyses could be repeated with more recent data to see if these relationships have continued or changed over time. Smoking prevalence has decreased in recent years; however, vegetable consumption has not improved.

To better understand the long-term impact of ETS exposure on chronic disease risk, a longitudinal study is warranted. A longitudinal study would increase support for the finding that nutrient biomarkers partially mediate the relationship between ETS exposure and metabolic syndrome score. While this study provides evidence that this relationship may exist, in order to prove causality, temporality is a critical missing link. If a study first examined ETS exposure, then later measured nutrient biomarkers, and finally later assessed metabolic syndrome risk and still found evidence of mediation, this would suggest that one of the mechanisms by which ETS impacts chronic disease risk is through creating an imbalance between nutrient concentration and biological processes requiring those nutrients.

The lack of statistically significant interaction in the third paper does not necessarily mean that dietary intake/quality do not modify the relationship between ETS exposure and metabolic syndrome risk. Because dietary intake of micronutrients and nutrient biomarkers are not perfectly correlated, it is possible that the adolescents in this

sample who had higher intake of the micronutrients being examined did not, in fact, have higher nutrient biomarker concentration.

These papers made new contributions to the literature with respect to ETS exposure, nutrient biomarkers, dietary intake/quality, and chronic disease risk. This work has identified a sub-group of adolescents who are potentially at higher risk for chronic disease due to multiple risk factors; determined that the negative effects of ETS exposure on chronic disease risk are evident as early as adolescence; provided support for the theory that one of the mechanisms by which ETS exposure affects chronic disease risk is through changes in nutrient biomarkers and biological processes that depend on those nutrients; and initiated investigation into the potential for dietary intake to impact the relationship between ETS and chronic disease risk. These findings support strict regulation of ETS exposure to adolescents and children, addition of dietary intervention to interventions targeting ETS-exposed adolescents, and further investigation to determine whether or not increasing micronutrient intake, via diet or supplement use, can counteract some of the harmful effects of ETS exposure.

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RESEARCH AND PRACTICAL EXPERIENCE

Research Scientist, 2013 to present, Battelle, Baltimore, MD.

Researcher, December 2010 to 2013, Battelle, Baltimore, MD.

Study Coordinator, 2009 to 2010, School of Medicine, Department of Pediatrics, Growth
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Teaching Assistant, 2008-2009, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD.

Summer Fellow, 2007, National Institutes of Health, National Heart, Lung, and Blood Institute, Bethesda, MD.

Program Evaluation Assistant, 2006–2007, Johns Hopkins University, The Center for Injury Research and Policy, Baltimore, MD.

Health Promotion Intern, 2004–2006, American University, The Wellness Center, Washington, DC.

Teaching Assistant, 2005–2006, American University, Health Promotion Department, Washington, DC.

Project Leader, 2006, American University, Wellness Project Team, Cooking in the Dorms, Washington, DC.

Campus Campaign Director, 2005–2006, National Council of Women's Organizations and Eating Disorders Coalition Extreme Measures Tour, Washington, DC.

Health Promotion Business Intern, 2005, XLHealth, Baltimore, MD.

Research Assistant, 2004, American University, Psychology Department, Washington, DC.

Research Assistant, 2003, University of Maryland, Maryland Center for Anxiety Disorders, College Park, MD.

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Dietetic Internship in Public Health Nutrition. Johns Hopkins Bayview Medical Center, 2007–2008

PhD coursework. Johns Hopkins Bloomberg School of Public Health. January, 2008-December, 2007

Certificate of Training in Child and Adolescent Weight Management, Commission on Dietetic Registration. Academy of Nutrition and Dietetics (formerly American Dietetic Association), 2008

Johns Hopkins Leadership Education in Adolescent Health Nutrition Fellowship. Department of Health and Human Services, 2008–2009

Region 3 Maternal and Child Health Leadership Skills Development Workshop, Johns Hopkins Bloomberg School of Public Health, 2009

National Research Initiative Human Nutrition and Obesity Workshop, 2009

Pediatric Academic Societies, 2009

The Obesity Society Annual Conference, The Obesity Society, 2010

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Academic & Research Ethics at JHSPH, Johns Hopkins Bloomberg School of Public Health, 2013

Webinar: Consumption of Cereal Fiber, Bran and Whole Grains and Risk of Chronic Diseases, American Society for Nutrition, 2014

Maryland Academy of Nutrition and Dietetics Annual Meeting, Maryland Academy of Nutrition and Dietetics, 2015

AWARDS

2013 – Outstanding Performance Award for significant contributions to the [Healthy Communities Study] HCS project, Battelle

2009 - Academy of Nutrition and Dietetics (formerly American Dietetic Association) Foundation Scholarship

2008 - Adele Diaz Departmental Scholarship, Johns Hopkins Bloomberg School of Public Health, Department of International Health, Human Nutrition

2007-2008 – Maryland Higher Education Commission, MD State Scholarship

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2006 – Magna Cum Laude, American University, Washington, DC

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SOCIETIES/ORGANIZATIONS

Journal of Physical Activity and Health, reviewer, 2012

American Public Health Association, 2011—Present

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PRESENTATIONS AND PUBLICATIONS

Sroka CJ, McIver KL, **Sagatov** RDF, Arteaga S, Frongillo EA. Weight Status Measures Collected in the Healthy Communities Study: Protocols and Analyses. American Journal of Preventive Medicine. 2015;49(4):642–646.

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Foreman RD. Nutrition in disease treatment and prevention: Cardiovascular disease. Invited speaker at the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, April 2010.

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Foreman RD. Adolescent Medicine Grand Rounds: Living up to our Challenge! Johns Hopkins Medical Institutions, Baltimore, MD, 2009.

Foreman RD. Leadership Education in Adolescent Health: Nutrition fellow update. Johns Hopkins Medical Institutions, Baltimore, MD, 2009.

Foreman RD. Youth Fitness Circle: Leadership education in adolescent health. Johns Hopkins Medical Institutions, Baltimore, MD, 2009.

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